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**Institutionen för kliniska vetenskaper Danderyds sjukhus**

# Effects of lignocaine in endometriosis- a clinical and cellular investigation

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# EFFECTS OF LIGNOCAINE IN ENDOMETRIOSIS – A CLINICAL AND CELLULAR INVESTIGATION

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# ABSTRACT

**BACKGROUND;** Endometriosis is a chronic inflammatory disease of unknown origin that can cause severe dysmenorrhea, chronic pain and impaired quality of life. Lignocaine has anti-inflammatory properties and pertubation with lignocaine could be beneficial in the treatment of endometriosis-associated pain. The Endometriosis Health Profile-30 (EHP-30) questionnaire is specific for endometriosis and evaluates quality of life. One scale on the EHP-30 core questionnaire has earlier failed to demonstrate any responsiveness.

**OBJECTIVE;** The objectives of the included studies were to evaluate the effect of pertubation with Ringer-Lignocaine on dysmenorrhea and quality of life in women with endometriosis. The safety and the pharmacokinetics of lignocaine after pertubation were to be investigated. Another objective was to evaluate the responsiveness of the EHP-30 questionnaire in a Swedish sample. In the final study, the objective was to evaluate the effect of lignocaine on cytokine expression and secretion *in vitro* in peritoneal fluid macrophages and endometriotic stromal cells.

**DESIGN;** Study I and III were double-blinded, randomised and controlled trials. Study II and IV were prospective observational studies and the fifth study was an experimental *in vitro* study on human cells.

**SETTING;** The studies were performed at three gynaecological outpatient units in Stockholm, Sweden and at the research laboratory at Uppsala Akademiska Hospital, Sweden.

**POPULATION;** Eligible patients had endometriosis as diagnosed by laparoscopy, dysmenorrhoic pain > VAS 50 mm (visual analogue scale) and patent Fallopian tubes. 42 women were included in study I, III and IV and of these, 25 were also included in study II. Peritoneal fluid and samples from endometriotic cysts were collected from 15 women (patients with endometriosis n=9, and healthy controls n=6) during surgery for clinical reasons in study V.

**METHODS;** The participants in study I-IV were randomised sequentially to pre-ovulatory pertubations with placebo (n=18) or lignocaine 1.0 mg/ml (n=24) during three consecutive menstrual cycles. The pertubation procedure comprised passing study solution through the uterine cavity and the Fallopian tubes via an intracervical placed balloon catheter.

In study I, the effect on pain was evaluated using a VAS scale before and after the treatments and up to nine menstrual cycles after the last pertubation. Success was defined as a reduction of pain ≥ 50% on the VAS scale. Fisher's exact test was used to compare the success rates between the treatment and the placebo groups.

In study II, serum samples were collected at 0, 5, 15 and 30 minutes after pertubation. The serum samples were analysed for the concentration of lignocaine with a LCMS-SIM method.

The effect on quality of life was evaluated with the EHP-30 questionnaire before and after the treatments in study III. The changes in scores from baseline to follow-up at six and twelve months were compared between the lignocaine and placebo groups with Mann Whitney U test.

In study IV, the changes on the EHP-30 questionnaire after three pertubations were compared with the patients' self-estimated change in pain intensity. The responsiveness to change for the EHP-30 questionnaire was evaluated with effect sizes and significance of change (paired t-test). The change in scores between those who improved (improved group) and those who did not improve (stable group) were compared with independent t-test.

In study V, macrophages from the peritoneal fluid and cells from the inside of the endometriotic cysts capsules were isolated and cultivated for 24 h-48 h in medium with and without the supplement of lignocaine to a final concentration of 0.1 mg/ml or 1.0 mg/ml. Relative gene expression of MCP-1, IL-6 and IL-8 were evaluated with RT-PCR and compared between treated and untreated cells with Wilcoxon matched pairs. The concentration of MCP-1, IL-6

and IL-8 in cell culture media was measured using ELISA and were compared between treated and untreated cells with Wilcoxon matched pairs.
**RESULTS;** Study I; In the Intention To Treat analysis in study I, the success rate was 41.7 % (10 of 24) in the treatment group compared to 16.7 % (3 of 18) in the placebo group (p=0.10). In the Per Protocol analysis, the proportion of subjects with success in the treatment group was 45 % (9 of 20) compared to 7.1 % (1 of 14) in the placebo group (p=0.024). Of the nine patients in the lignocaine group that fulfilled the criteria for success after three pertubations, four had an effect persisting after nine months.
Study II; Low levels of lignocaine were detected in the serum samples following pertubation of 10 mg lignocaine hydrochloride. The highest observed concentration was seen after 30 minutes with a mean of 0.050 µg/ml and an individual maximum of 0.124 µg/ml.
Study III; After six months there was a significant difference between the lignocaine (n=19) and the placebo (n=16) groups on the dimension social support (median -18.8 vs. -6.3, p=0.034) whereas there were no differences for the other dimensions after six or twelve months.
Study IV; The change in scores were significant for all dimensions (p=0.04-0.0002) except sexual intercourse (p=0.29) for improved patients in contrast to the patients in the stable group where there were no significant changes in any dimension (p=0.16-0.63). The effect sizes were large (>0.8) on all core scales except self image (0.51) for the improved patients and small on all scales in the stable group (-0.17-0.35). There were significant differences between the improved and the stable group considering change in most of the core EHP-30 scores.
Study V; The gene expression and protein secretion of IL-8 in endometriotic stromal cells after incubation with lignocaine 0.1 mg/ml was significantly decreased after 24 h compared to control (p=0.03 and p=0.02). Macrophages from healthy controls had a significantly lower gene expression of all tested cytokines (p=0.04) after treatment with lignocaine but there were no significant differences on protein level. Macrophages from patients with endometriosis (n=5) showed diverging results since three samples showed increased gene expression of one or two cytokines after lignocaine treatment.
**CONCLUSIONS;** The first study indicates that pertubation with lignocaine is an efficient, non-hormonal treatment option for some patients with dysmenorrhea and endometriosis. The serum levels of lignocaine following pertubation of 10 mg lignocaine hydrochloride are detectable but low. Pertubation with lignocaine is safe and have no adverse events related to lignocaine. Study III indicates that pertubations with lignocaine might improve the social support dimension of quality of life in patients with endometriosis. EHP-30 is responsive to improvement on all scales on the core questionnaire and is acceptable, understandable and applicable in our Swedish sample. Lignocaine can affect the gene expression and secretion of the pro inflammatory cytokine IL-8 *in vitro*. Macrophages from patients with endometriosis might be dysregulated. The data presented in this thesis indicate that due to differences at cellular level, nearly half of the endometriosis population may benefit from lignocaine pertubations.

## LIST OF PUBLICATIONS

I. **Wickström K**, Bruse C, Sjösten A, Spira J, Edelstam G. **Pertubation with lignocaine as a new treatment of dysmenorrhea due to endometriosis: a randomized controlled trial.** Hum Reprod. 2012 Mar; 27(3):695-701

II. **Wickström K**, Spira J, Edelstam G. **Serum Concentration of Lignocaine After Pertubation: An Observational Study.** Drugs R D. 2013 Sep;13(3):235-9

III. **Wickström K**, Bruse C, Sjösten A, Spira J, Edelstam G. **Quality of life in patients with endometriosis and the effect of pertubation with lidocaine -a randomized controlled trial.** Acta Obstet Gynecol Scand. 2013 Dec; 92(12):1375-82

IV. **Wickström K**, Spira J and Edelstam G. **Responsiveness of the Endometriosis Health Profile-30 questionnaire in a Swedish sample- an observational study**. Submitted

V. **Wickström K**, Stavréus-Evers A, Vercauteren O, Olovsson M and Edelstam G. **Effect of lignocaine on IL-6, IL-8 and MCP-1 in cultures of peritoneal macrophages and endometriotic stroma cells from women with endometriosis.** Manuscript

# CONTENTS

# LIST OF ABBREVIATIONS

| | |
|---|---|
| AE | Adverse events |
| AI | Aromatase inhibitors |
| APL | Apoteket Production & Laboratories |
| ASRM | American Society of Reproductive Medicine |
| ART | Artificial reproductive technologies |
| BMI | Body Mass Index |
| BrDU | 5-bromo-2-deoxyuridine |
| CA-125 | Cancer antigen 125 |
| cDNA | Copy deoxyribonucleic acid |
| Cmax | Maximum concentration |
| CNS | Central nervous system |
| CRP | C reactive protein |
| CT | Cycle threshold |
| CONSORT | Consolidated Standards of Reporting Trials |
| ΔΔCT method | Delta-Delta-Cycle Threshold-method |
| ECC | Endometriotic cyst capsule |
| EHP-30 | Endometriosis Health Profile-30 |
| ELISA | Enzyme-linked immunosorbent assay |
| ES | Effect size |
| G-CSF | Granulocyte colony stimulating factor |
| GnRH | Gonadotropin releasing hormone |
| HLA | Human leukocyte antigen |
| HRQL | Health related quality of life |
| IQR | Inter quartile range |
| NK cell | Natural killer cell |
| IL-1 | Interleukin-1 |
| IL-6 | Interleukin-6 |
| IL-8 | Interleukin-8 |
| ITT | Intention to treat |
| IVF | In vitro fertilisation |
| KATP channels | ATP-sensitive potassium channels |
| LA | Local anaesthetic |
| LCMS-SIM | Liquid Chromatography - Mass Spectrometry simulator |
| LNG-IUS | Levonorgestrel intrauterine system |
| LUNA | Laparoscopic uterine nerve ablation |
| MØ | Macrophages |
| M-CSF | Macrophage colony-stimulating factor |
| Max | Maximum |
| MCP-1 | Monocyte chemotactic protein-1 |
| MID | Minimal important difference |
| Min | Minimum |
| MPA | Medroxyprogesterone acetate |
| mRNA | Messenger ribonucleic acid |
| MWU test | Mann Whitney U test |
| NSAIDs | Non-steroidal anti-inflammatory drugs |

| | |
|---|---|
| NRS | Numerical/numeric Rating Scale |
| OC | Oral contraceptives |
| PBS | Phosphate buffered saline |
| PGE2 | Prostaglandin E2 |
| PGF2 | Prostaglandin F2 |
| PP | Per protocol |
| PRO | Patient reported outcomes |
| QOL | Quality of life |
| QPCR | Quantitative polymerase chain reaction |
| RANTES | Regulated on Activation, Normal T Cell Expressed and Secreted |
| RCT | Randomised controlled trial |
| RNA | Ribonucleic acid |
| RT-PCR | Realtime PCR |
| SD | Standard deviation |
| SF-36 | Short form -36 |
| SRM | Standardised response mean |
| TNF-α | Tumor necrosis factor-α |
| Tmax | Time to maximum concentration |
| VAS | Visual analogue scale |
| VEGF | Vascular endothelial growth factor |

# 1 INTRODUCTION

Von Rokitansky first described endometriosis in 1860. There are 176 million women suffering from endometriosis worldwide, an invisible disease causing considerable distress for affected women and high costs for society (1). Endometriosis is a complex disease and a lot of research has been conducted aiming to understand the pathophysiology and thereby find effective treatment options. No cure is presently available for this chronic disease and previous therapies aiming to relieve the symptoms have clear deficiencies (2). The studies presented in this thesis aim to investigate a potential new treatment for patients with endometriosis-associated pain.

## 1.1 BACKGROUND ENDOMETRIOSIS

### 1.1.1 Definition

Endometriosis is defined by the presence of endometrium-like tissue with glands and stroma located at aberrant locations outside the uterine cavity. The ectopic endometrium is implanted over the visceral and peritoneal surfaces in the lower pelvis but also deeper locations of endometriotic tissue in the ovaries (endometriotic ovarian cysts) are common. In addition, endometriotic implants can infiltrate the rectovaginal septum (adenomyosis externa) and the myometrium (adenomyosis interna). Extensive intrapelvic adhesions may occur, especially in cases of ovarian endometriosis. Adhesions, fibrosis and scarring around endometriotic implants and cysts may distort the anatomy and occlude the fallopian tubes (3). Endometriotic lesions can even appear at extra genital locations with the intestinal tract being the most common site, constituting 5% of all endometriosis. Other locations in which endometriosis can occur is the urinary tract, in scars in the abdominal wall, in the inguinal canal and in the thorax (4).

### 1.1.2 Prevalence and diagnosis

Endometriosis affects 6-10% of all fertile women and up to 35-50% of females with dysmenorrhea and/or infertility, which are the main symptoms of endometriosis (5-8). At menopause, the lesions usually disappear and the pain vanishes.

Laparoscopy or laparotomy with visualisation of endometriotic implants or cysts can confirm the diagnosis (8, 9). Operative visualisation of characteristic lesions is generally considered an acceptable surrogate for excision with histologic diagnosis of endometriosis (10). The lesions can be opaque and nearly invisible, red, brown or white (10, 11). The red lesions seem to be the most active form (11). Follow up of women with pelvic pain and endometriosis has shown that 17-29 % of lesions resolve spontaneously, 24-64 % progress and 9-59 % are stable over a 12 month period (8). The revised scoring system of the American Society of Reproductive Medicine (ASRM) is used to determine the disease stage. The score is ranging from I, indicating minimal disease, to IV, indicating severe disease taking into account the presence and size of ovarian endometriotic cysts and extent, type, and site of peritoneal lesions.

The diagnosis is often delayed and the mean interval between the onset of pain and diagnosis is 10.4 years (8, 12). After years of pain women feel relieved when a diagnosis has been confirmed (12). Transvaginal ultrasonography and magnetic resonance imaging (MRI) can

reliably detect extragenital endometriosis and endometriotic cysts (8, 9) but is unreliable in the diagnosis of peritoneal non-ovarian endometriosis (13).
Efforts have been made to find a non-invasive diagnostic tool to detect peritoneal endometriosis. CA-125 may be elevated in serum in patients with endometriosis, but has poor sensitivity and specificity and is not recommended for diagnostic purposes (8). Also auto-endometrial antibodies and integrin-proteins have been proposed as biomarkers for non-surgical diagnosis of endometriosis but have failed to prove valuable (11).
Endometrial biopsy and immunohistochemical nerve fibre detection might be a new and less invasive diagnostic test. Women with endometriosis and pelvic pain have fine, non-myelinated nerve fibres present in the functional layer of the endometrium (14) and the density of small nerve fibres is 14 times higher in endometrium from patients with endometriosis compared to healthy controls (15).

### 1.1.3 Symptoms

Pain in the lower abdomen and subfertility/infertility are the main symptoms of endometriosis. The first symptom is most often dysmenorrhea. Other symptoms that may occur over time is non-menstrual pelvic pain, deep dyspareunia, dyschezia, chronic pelvic pain and impaired fertility (16). The pain can occur intermittently throughout the menstrual cycle or be continuous. Furthermore, the pain can be dull, throbbing or sharp and the pelvic pain is usually chronic (lasting > 6 months) (8). The degree of pain is not related to the severity as classified by ASRM (8, 17, 18). Neither is there a correlation between pain severity and endometriotic ovarian cysts (18).
Women with endometriosis have a higher incidence of depression and anxiety compared to women without endometriosis, and the severity is related to the pain intensity (19-22). Depression has proved to be a consequence of the present chronic pain but predisposition to depression may increase the likelihood (22).
Endometriosis patients have impaired health related quality of life (HRQL) compared to women without endometriosis (19, 23, 24) and even worse than women with depression (25). As with depression, the impairment is related to the degree of pain (19, 26) and women with chronic pelvic pain report worse HRQL compared to healthy women (24). Moreover, the decreased fertility can affect quality of life since infertile women have lower HRQL scores compared to both infertile men and normative data (27). As chronic pelvic pain and infertility are the most severe symptoms and consequences of endometriosis, they have the highest impact on HRQL in endometriosis patients (2).
Among women with endometriosis, 30-50% is infertile (3, 11, 28). Infertility is defined as the inability to conceive after one year of unprotected intercourse (11) and 25-40% of women with infertility have endometriosis (11, 16). In normal couples the probability of achieving a pregnancy in any single month is 15-20% whereas it in couples with an untreated women with endometriosis is less than 5% and depends on the severity of the disease (11).

### 1.1.4 Pathogenesis

The understanding of the pathogenesis and the pathophysiology of the disease remains unclear (29). Three theories have been proposed to explain the mechanism behind the development of endometriosis (30).

The implantation theory (Sampson 1927) includes implantation and growth of endometrium following retrograde menstrual reflux. The other two theories suggest that the endometrial tissue originate from the peritoneal serosa or from derivates of the Müllerian ducts. The theory of coelomic metaplasia (Meyer 1919) proposes that the germinal epithelia of the ovary could be transformed by metaplasia into endometrium (30).

There are convincing evidence supporting all theories but the reflux implantation theory is the most widely accepted. The anatomical distribution of implants follows the distribution of menstrual reflux. The endometrium fragments reaching the peritoneal cavity are viable and can attach to the peritoneum. They can degrade the basal membrane, produce angiogenic factors essential for neovascularisation and thereby implant in the peritoneal surfaces (30, 31).
The reflux of menstrual debris seems to be necessary but not sufficient for the development of endometriosis. Retrograde menstruation can be seen as a physiologic phenomenon in 76-90% of all women (29, 32, 33) but only around 10% develop endometriosis. In most women the peritoneal environment is capable of resorbing the menstrual debris from the peritoneal cavity at the end of menstruation, preventing it to adhere to the peritoneal surfaces. In patients developing endometriosis the cleaning system seems to be insufficient. Immunologic changes, genetic factors and environmental factors may determine the susceptibility to develop endometriosis (34). A composite theory of retrograde menstruation with implantation of endometrial fragments in conjunction with immunological and peritoneal factors is the most widely accepted explanation for endometriosis (28, 34, 35).

It has been proposed that the mechanism behind the development of ovarian endometriosis and deep endometriosis is different from the development of superficial implants (5, 26, 36). It has been suggested that superficial endometriotic implants located on the ovarian surfaces could be invaginated or a functional ovarian cyst could be secondary involved by endometrial implants on the peritoneal surface (5). In addition, the mesothelium covering the ovary could invaginate and undergo coelomic metaplasia and subsequent formation of endometriotic cysts (5, 36). Deep endometriotic lesions in the rectovaginal septum might be an evolution of peritoneal endometriosis of the pouch of Douglas secondary to infiltration (5). The fact that 93.5% of cases with deep endometriosis also have superficial implants, endometriotic ovarian cysts and/or pelvic adhesions, support this theory (5).

Thus, epidemiological, surgical and pathological data consistently suggest that peritoneal, ovarian and deep lesions constitute different expressions of one single disease with a unique pathogenic mechanism i.e. retrograde menstruation (5). Hereditary factors, immunological environment and local hormone concentrations in peritoneal fluid or ovary will determine whether the endometrial implants will develop into typical lesions, deep endometriosis or cystic ovarian endometriosis (30, 31). High concentrations of oestradiol are needed for the development of endometriotic cysts and are found only inside or in the proximity of the ovary (30, 31). Superficial endometrial implants are regulated by peritoneal fluid factors, deep endometriosis is affected by blood factors and cystic ovarian endometriosis by ovarian factors (31).

### 1.1.5 **Risk factors**

Endometriosis may occur if the amount of tissue is too great or if the capacity of the intra-abdominal cells to clear the abdominal cavity is impaired. Excessive menstruation or an obstructive uterine anomaly, which increases the menstrual reflux, increases the risk for endometriosis (5, 28, 37-39). Women with early menarche, late menopause and nulliparous have an increased risk (5) whereas prolonged lactation and multiple pregnancies are protective (8). The risk also seems to be higher with increasing age (5).
The genetic factor in the aetiology of endometriosis is strong (5, 30, 31) and the risk of endometriosis is seven-fold increased in women with first degree relatives having endometriosis (40). Endometriosis is associated with higher risks of autoimmune diseases and ovarian endometrioid and clear-cell cancers, as well as other cancers, including non-Hodgkin's lymphoma and melanoma (8).
Various exposure factors, for example dioxin, are thought to be associated to endometriosis by stimulating the production of pro-inflammatory cytokines (41).

### 1.1.6 **Pathophysiology**

The endometrium, the immune system, the peritoneum and the fallopian tubes are all suggested factors to be involved in the pathophysiology of endometriosis.

#### 1.1.6.1 *Endometrium*

The eutopic endometrium differs between women with and without endometriosis and has a higher capacity to implant and grow on peritoneal surfaces (30). Factors favouring the implantation of the endometrium have been identified for all processes potentially involved in the phenomenon. Molecules involved in apoptosis and adhesion, growth and angiogenic factors, matrix metalloproteinases and mechanisms involved in the escape from the immune system have all been recognized as qualitatively or quantitatively different in eutopic and/or ectopic endometrium of women with endometriosis compared to the endometrium of disease-free women. These alterations might affect the physiological activity of endometrium and are thought to explain why only some women develop the disease (5).
Molecular alterations between the endometriotic lesions and the eutopic endometrium in women with endometriosis have been found. These can be constitutive and/or acquired since the pathological peritoneal and ovarian environment in women with endometriosis might modify a normal endometrium (5, 31).
Other abnormalities found in endometriotic implants and in eutopic endometrium of endometriotic patients include abnormal expression of aromatase and a progesterone resistance (6).

#### 1.1.6.2 *Immunological changes*

There is substantial evidence that immunological factors play a decisive role in the development and pathogenesis of endometriosis (38, 42). Observed immune alterations include increased number and activation of peritoneal macrophages, decreased T-cells reactivity and NK cell cytotoxicity, increased autoantibodies and changes in the cytokine network (29). Immunological changes may determine the ability of endometrial implants to

survive in ectopic locations and thereby the susceptibility of a woman to endometriosis (29, 32). Whether the anomalies may contribute to the development of endometriosis or are a consequence of the disease has not been resolved (28-30, 33).
It is agreed that a local sterile and chronic inflammation occurs in the peritoneal cavity in patients with endometriosis (42). Macrophages appear to play a leading role in the process but other cell types contributing to the inflammatory process are peritoneal mesothelium, lymphocytes and the endometrium itself. A complicated network of different cell types and their secretory products interact locally and can regulate cell proliferation, activation, motility, adhesion, chemotaxis and morphogenesis in many different cell types (28, 38, 43). The peritoneal inflammation can in this way affect the implantation, growth, invasiveness, neovascularisation and maintenance of the endometrial implants and may also give rise to symptoms such as pain, infertility and adhesions (28, 30).
Elevated serum levels of CRP and CA-125 indicate a generalized inflammatory activity that in some patients give symptoms such as fever and a general feeling of malaise, especially during periods with more pain (44).

#### 1.1.6.2.1 The role of the peritoneal fluid

The peritoneal fluid (PF) in women with endometriosis contains increased amount of immunologic cells and inflammatory mediators such as cytokines, prostaglandins and growth factors (28, 41, 45). The cellular constituents include macrophages, NK cells, lymphocytes, eosinophils, mesothelial cells and mast cells. The PF also contains steroid hormones, prostaglandins, cytokines and growth factors (28, 42). It arises primarily from plasma transudate and ovarian exudate but other sources are tubal fluid, retrograde menstruation and macrophage secretions (28). The volume of PF is usually around 5-20 ml but varies widely during the menstrual cycle and between individuals (28, 31). The volume of PF in women with endometriosis might be modestly increased (28, 42).
The PF is a specific microenvironment and the concentration of soluble constituents around endometriotic implants might be higher especially if accompanied by adhesions, due to compartmentalization (31). Ovarian steroid hormone concentrations are 10-100 fold higher in PF than in plasma (31). The PF surrounding the endometriotic implant is dynamic (42) and have a large exchange area with plasma through peritoneum (31).

#### 1.1.6.2.2 Cell-mediated immunity and endometriosis

*Macrophages* constitute 85% of the cells in the peritoneal fluid (42, 45) and probably represent the first line host response to an inflammatory stimulus (28). Macrophages digest and process peritoneal debris such as spermatozoa and endometrial tissue and present antigens to T-cells. In addition they secrete various substances such as growth factors, cytokines, angiogenic factors, prostanoids, complement components and hydrolytic enzymes (28, 30, 34). Products from macrophages can effect both immune and non-immune cells (29) and give rise to a chronic inflammation in the peritoneal cavity (30).
In women with endometriosis the peritoneal macrophages are increased in total number, concentration and activational status and some data suggest that they might even be more differentiated (28, 34, 46). The presence of endometrial cells in the peritoneal fluid enhances monocyte recruitment and activation (47). Macrophage products are increased in patients with endometriosis and can play a role in the initiation, maintenance and progression of

endometriosis (28, 34, 48) by affecting the survival and growth of ectopic endometrial cells (28, 29, 34).
An increased sperm phagocytosis by peritoneal macrophages has been found in patients with endometriosis (49). Moreover, the scavenger receptor function in the macrophages might be defective and thereby their function to recognize cellular debris (29).
*Natural killer (NK)* cells have been suggested to play a role in the clearance of regurgitated endometrial cells in the peritoneal cavity and women with endometriosis have NK cells with decreased cytotoxicity (29). This may increase the likelihood of implantation (34). NK cell activity is decreased in both peripheral blood and in PF of patients with endometriosis and the activity level is inverse related to the stage of the disease (42, 50).
*T-cells* facilitate the cell-mediated immune response and their cytokines can affect B-cells, macrophages, NK cells and other T-cells. An increased T-helper to T-suppressor ratio has been noted in the PF in women with endometriosis, suggesting an impaired cellular immune activity (38). The data is inconsistent for changes in T cells concentration (28).

1.1.6.2.3 Humoral immunity and endometriosis

In patients with endometriosis there is evidence for alterations in B-cell activity and an increased incidence of auto-antibodies has been found in both serum and PF (29, 34). The increased immune autoimmunity may be due to enhanced reactivity to normal self-antigens because of a genetic predisposition or an excess of endometrial antigens in the peritoneal cavity (34). Although a strong genetic component exists in endometriosis, association with a specific HLA haplotype has not yet been demonstrated and the definition of endometriosis as an autoimmune disease has not been manifested (29, 34).

1.1.6.2.4 Cytokines

Cytokines are postulated to participate in the pathogenesis of endometriosis (28). The cytokines are multifunctional proteins with large properties and can be produced by peritoneal macrophages, lymphocytes, ectopic endometrial implants (epithelial and stromal cells) and from mesothelial cells lining the peritoneum (38).
Cytokine activities include; proliferation and differentiation of immune cells; growth of connective tissue and endothelial cells; induction of release of hormones, enzymes, acute phase proteins and other cytokines; enhancement of various cytotoxic activities; regulation of immunoglobulin secretion and chemotaxis (28, 38, 42). Cytokines exert effects on a variety of cell types and act as key mediators of intercellular communication within the immune system (29).
Patients with endometriosis have higher levels of several pro inflammatory cytokines in the PF, for example IL-1, IL-6, IL-8, MCP-1, TNF-α, VEGF and RANTES (29, 34, 38, 42, 51-53).
*TNF-α (Tumour necrosis factor-α)* is secreted from PF macrophages (28, 34). It enhances the adhesion of endometrial stromal cells to mesothelial cells, is embryotoxic and may affect sperm motility (28, 34). The level of TNF-α in PF is positively correlated to the level of menstrual pain (54) and the level decreases after medical treatment (53).
*IL-1 (Interleukin-1)* induces prostaglandin synthesis, T-cell proliferation and stimulates B lymphocytes (28, 34). It may also have stimulatory effect on fibroblast proliferation and

collagen deposition suggesting a role in the pathogenesis of adhesions and peritoneal fibrosis in endometriosis (28, 34). The level of IL-1 in PF decreases after medical treatment (53).
*VEGF (vascular endothelial growth factor*) is a potent angiogenic factor and activated macrophages are the major source of VEGF (55). The expression is regulated by ovarian steroid hormones (31, 56).
*RANTES (Regulated on Activation, Normal T Cell Expressed and Secreted)* is produced by ectopic endometrium and is a selective chemo attractant for monocytes and T-cells (57). The concentration of RANTES is related to the severity of the disease (28, 34, 58).
*IL-6 (Interleukin-6)* is secreted by macrophages, endometrial cells, ectopic endometrial tissue (both epithelial and stromal cells), lymphocytes, monocytes, fibroblasts, endothelial cells and keratinocytes (38, 42, 59). IL-6 acts on macrophages, B-cells and T-cells and regulates immune response by modulating the secretion of other cytokines and by acting as a growth regulator (29, 42). It stimulates angiogenesis, promotes proliferation and is related to tissue repair (28, 34, 38, 42, 60). Peritoneal fluid levels of IL-6 correlate well with the severity of endometriosis (29, 61, 62). Increased concentrations of IL-6 have been found in ectopic endometrial tissue culture of women with endometriosis (42, 53).
*IL-8 (Interleukin-8)* is produced by monocytes, endothelial cells, fibroblasts, mesothelial cells and endometrial stromal cells (28, 29, 38). It stimulates angiogenesis, inflammation and proliferation in numerous cells including endometrium and endometriotic stromal cells (28, 38, 42, 48, 51, 63). The levels of IL-8 in the peritoneal fluid are correlated with the severity of endometriosis (38, 64). IL-8 is thought to play a role in the pathogenesis and maintenance of endometriosis by inducing the attachment of endometrial cells to fibronectin (38, 57) and by acting as an autocrine growth factor in the endometrium (29).
*MCP-1 (Monocyte chemotactic protein-1)* is secreted by endothelial cells, mesothelial cells, monocytes/macrophages, fibroblasts, leukocytes and by both epithelial and stromal cells in endometrial tissue (29, 65-68). It is a potent chemotactic and activating factor for monocytes and attracts, activates and stimulates macrophages to secrete growth factors and cytokines (28, 42, 65, 69) but also stimulate endometrial cell proliferation (42, 69). The levels of MCP-1 in the PF are correlated to the severity of the disease and medical treatment with gonadotropins suppresses the concentrations (69).

#### 1.1.6.3 *The role of peritoneum*

The peritoneum can be more or less receptive to implantation of endometrium and the risk for development of endometriosis could to some part depend on peritoneal factors (30). The peritoneum consist of a thin layer of connective tissue covered by a layer of mesothelium (42) and is highly permeable for small molecules. The total surface area is around 2 $m^2$ or about equal to that of the skin (28, 31). The mesothelial cells of the peritoneum produces IL-1, IL-6, IL-8, MCP-1, G-CSF, M-CSF and CA-125 and are suggested to play a role in the regulation of peritoneal inflammation and tissue regeneration (28).

#### 1.1.6.4 *The fallopian tubes*

Women with endometriosis have a uterine hyperperistalsis that differs significantly from the peristalsis of healthy controls. The defect uterine contractions can participate in the pathogenesis of endometriosis by increasing the risk for retrograde menstruation but even be an explanation for the impaired fertility since the sperm transport might be defect (70).

#### 1.1.6.5 *Pathophysiology behind symptoms*

##### 1.1.6.5.1 Pain

The pain in patients with endometriosis can be of several different kinds, each of which might have different pathophysiology. The explanation of the increased pelvic pain involves inflammatory substances and increased density of sensory nerves fibres in the endometriotic lesions and the eutopic endometrium (71-73). Effects of active bleeding from endometrial implants and anatomical distortion caused by adhesions and fibrosis have also been suggested (8, 10, 16).

Peritoneal endometriotic lesions may cause all symptoms but deep infiltrating endometriosis and extensive adhesions may cause more severe pain (17, 31). Correlations have been found between pain severity and both the depth of infiltration into peritoneum or pelvic organs and the pro-inflammatory substances released by the implants into the peritoneal cavity (10, 71). The pain level seems to be associated with the distance between nerve fibres and implants and the number of nerve fibres within lesions (74, 75). Increased amounts of sensory nerve fibres have been found in peritoneal endometriotic lesions and in the functional layer of the eutopic endometrium in patients with endometriosis compared to peritoneum and endometrium of healthy women (76, 77). Hormonal treatment with oral contraceptives (OC) or progestogens significantly reduced nerve fibre density in endometrium, myometrium and peritoneal lesions (78, 79). Another study showed correlation between the occurrence of nerve fibres in ovarian endometriotic lesions and pain intensity (80). In addition, women with endometriosis have a higher density of neuroendocrine cells in the endometrium (81). Thus, inflammatory substances and substances produced by neuroendocrine cells can sensitise and/or activate the sensory nerve endings (82) and contribute to the pain in endometriosis. A sensitisation of sensory nerve fibres can also produce a central sensitisation which is a long-lasting hyper excitability of neurons in the central nervous system (71).

##### 1.1.6.5.2 Subfertility

Distortion of the anatomy and/or adhesions causing occluded fallopian tubes are understandable factors causing infertility but mechanisms underlying reproductive failure in minimal endometriosis remain controversial (3). Mechanisms investigated include altered folliculogenesis, ovulatory dysfunction, reduced steroid production of granulosa cells, defects in luteal phase function and alterations within the oocyte (41). Other mechanisms investigated are sperm phagocytosis, anti-sperm antibodies, impaired fertilisation, decreased capability of fimbrial ovum capture, toxicity against early embryonic development and defective implantation (41).

Cytokines and the immunologic alterations in the PF are believed to be related to endometriosis-associated infertility and can affect all the above functions (11, 28, 29).

A reduced oocyte and embryo quality with impaired fertilisation, embryo cleavage and implantation rate has been found in patients with endometriosis (83, 84) and women with endometriosis have approximately 50% lower fertility frequency after IVF compared to women with tubal defect (3, 85, 86). Moreover, endometrial changes and an altered uterine and tubal contractility have been suggested to be involved in the endometriosis-associated infertility by affecting normal sperm transport and the implantation process (11, 83, 87).

An increased sperm phagocytosis by macrophages has been found in the PF of patients with infertility and endometriosis (49) and the PF also decreases the sperm velocity and the proportion of motile spermatozoa when added to medium *in vitro* (28, 29, 34). Abnormal autoantibodies are thought to play a role in the endometriosis-associated infertility by affecting the implantation (29, 34, 88).
Most data suggest that endometriosis affect fertility via the oocyte/embryo and not through the endometrium (3) since no differences in pregnancy, implantation and live birth rate were seen between healthy women and women with endometriosis who received oocytes from a single healthy donor (83).
The risk for miscarriage is not increased in women with endometriosis (11, 89, 90) but the rates of pregnancy loss after IVF are higher (6).

### 1.1.7 **Treatment**

There are several treatment options for women with endometriosis aiming to relieve pain and/or ameliorate infertility but none of them are curative (16). Pharmacological and surgical therapies are available, can be combined and can improve patients physical functioning, psychological functioning, vitality, pain level and general health (2). Surgical treatment followed by medical therapy offers longer symptom relief than surgery alone (8, 10).
Medical therapy can be initiated for pain control without surgical confirmation of endometriosis (8) and is recommended by both American College of Obstetricians and gynecologists and Royal College of Obstetricians and Gynaecologists (13).
The risk of pain recurrence after completion of treatment is high for all treatments and may be as high as 50% after 12-24 months (9). The stage (ASRM) or morphologic types of lesions do not predict the response to therapies for pain or infertility (8, 91).

#### 1.1.7.1 *Analgesics*

NSAIDs (non-steroidal anti-inflammatory drugs) suppress the synthesis of prostaglandins and can relieve dysmenorrhea (16). No significant reduction in pain due to endometriosis was shown in a small RCT when NSAIDs was compared with placebo and no superiority of one NSAID over another (8, 92).

#### 1.1.7.2 *Hormonal treatment of pain*

Hormonal treatments aim to lower the concentration of circulating oestrogen and thereby suppress the stimulatory effect on endometriotic lesions, leading to quiescence and regression of active lesions (16). All hormonal treatments suppress ovarian activity, reduce menstrual bleeding and induce decidualisation and atrophy of endometriotic implants at various extents (9, 10, 16). Complete down-regulation of ovaries and hypo-oestrogenaemia does not seem to be crucial and achievement of amenorrhea seems to be sufficient (93).
All hormonal treatments are contraceptive and include oral contraceptives, progestogens, androgenic agents and gonadotropin releasing hormone (GnRH) analogues (9). All hormonal treatments are similarly effective in relieving pain but the side effects differ (8, 9, 16, 94).
During hormonal treatment 60-100% of patients get pain relief (8, 9, 16, 94) but the chances for spontaneous pregnancy do not improve (8, 11). Endometriotic cysts are not amenable to medical treatment even if a temporary reduction in size may occur (9, 10).

#### 1.1.7.2.1 Combined oral contraceptives

Combined oral contraceptives (OC) can be used cyclically or continuously for pain related to endometriosis (10, 16). 75-80% of patients gets pain relief from OC and a continuous use can further improve the effect (8). Side effects include nausea, weight gain, fluid retention, depression, breakthrough bleeding, breast tenderness and headache (8). The treatment can be used long term and also the contraceptive ring or a transdermal patch can be used (9, 16).

#### 1.1.7.2.2 Danazol

Danazol acts primarily by inhibiting luteinizing hormone surge and steroid genesis and by increasing free testosterone levels (10). Danazol was an early treatment for endometriosis but its androgenic and anabolic side effects limit its clinical usefulness even if the effect on pain is comparable to other hormonal therapies (8). Side effects include weight gain, oedema, bloating, acne, lipid profile alterations, hirsutism and skin rashes (8, 9, 16). The treatment can be used for 6-9 months (9).

#### 1.1.7.2.3 Progestogens

Oral progestogens used for treatment of endometriosis include medroxy progesterone acetate (MPA, Provera®), Noretisteron (Primolut-Nor®), Desogestrel (Cerazette®), Dienogest (Visanne®) and Cyproterone acetate (16). MPA is the most used and the effect on pain equal with OC (8). The treatment can be long term and the inter-individual variation in dosage is large (95). The side effects include nausea, weight gain, bloating, depression, fluid retention, acne, headache, breast tenderness, irregular bleeding or breakthrough bleeding and the return of ovulation may be delayed after treatment has stopped (8, 9, 16).
The levonorgestrel intrauterine system (LNG-IUS) can be used long term and suppresses the endometrium and even the ovaries in some women (9). Similar effectiveness on pain as GnRH agonists has been shown (9, 16) but the exact mechanism is unknown since it does not inhibit ovulation and does not induce a hypo-oestrogenic state (in serum) (16). Postoperative treatment with the LNG-IUS after conservative surgery, diminish dysmenorrhea and non-cyclic pelvic pain but not dyspareunia (8, 9, 16). A significant decrease in the extent of disease has been found when observed at a second look laparoscopy after six months treatment with LNG-IUS (10, 16).

#### 1.1.7.2.4 Gonadotropin releasing hormone agonists (GnRH agonists)

GnRH agonists are a second line treatment after OC or progestogens have failed and can be used for 6 months (9, 16). They deplete the pituitary of endogenous gonadotropins and result in very low local and circulating oestrogen levels causing endometrial atrophy and amenorrhea (8, 9, 16). The side effects include vaginal dryness, hot flushes, psychiatric symptoms, decreased libido, irritability/mood swing, headache and reduced bone mineral density (8-10, 96). Add-back therapy with a low dosage oestrogen-progestogen is recommended to maintain bone mineral density and does not affect the efficacy on pain symptoms (8, 16). A follow up study of patients treated with GnRH agonists alone for six months revealed a 53% recurrence of disease/symptoms two years after treatment (10). The need for subsequent therapies was 37-74% five years after GnRH treatment (97).

#### 1.1.7.1 *Surgical treatment of pain*

Surgical procedures are indicated to alleviate pain symptoms (16). Procedures include excision, fulguration or laser ablation of endometriotic implants, excision or drainage of endometriotic cysts, resection of rectovaginal nodules, lysis of adhesions and interruption of nerve pathways (8). Laparoscopic destruction of endometriotic implants is associated with significant reduction in pain compared to diagnostic laparoscopy (8, 98, 99). Laparoscopic excision of invasive endometriosis improved pelvic pain, dysmenorrhea, dyspareunia, rectal pain but also significantly improved quality of life (23). One year after surgery the pain relief ranges between 50 and 95% (10) but pain recur within 6-12 months in 30-60% of patients (8, 94) and in up to 75% of women within 2 years after surgery (6). At laparoscopic follow up after one year, recurrent disease was revealed in 44% of patients (10). Surgical treatment followed by medical therapy (GnRH agonists, Danazol or OC) offers longer symptom relief than surgery alone (8, 10, 96).

The addition of LUNA (laparoscopic uterine nerve ablation) to laparoscopic surgical treatment is not associated with a significant difference in outcome (8, 10, 100). Pre-sacral neurectomy has proved to be superior to laparoscopic ablation alone for treatment of dysmenorrhea but there is insufficient evidence to recommend this treatment (101).

Surgery is the primary approach for symptomatic and/or large endometriotic cysts (10, 102) and excision/enucleation of the cysts has better long time effect on pain and lower recurrence rate compared to drainage and ablation (8, 10, 103).

A pain relief can be provided in 80-90% of women after hysterectomy with bilateral salpingo-oophorectomy but pain was reported to recur in 10% of the women within 1-2 years (8). Hysterectomy with bilateral salpingo-oophorectomy is more effective than hysterectomy alone (104). A hormone replacement therapy with both oestrogen and progesterone is recommended for patients severely suffering from post-surgical menopause to lower the risk for recurrence of endometriosis as compared to oestrogens alone (8, 10, 105).

#### 1.1.7.2 *Treatment of infertility*

Medical treatment (with OC, GnRH agonists, progestogens or Danazol) does not improve the chances for spontaneous pregnancy in women with endometriosis (8, 11, 16, 106).

Ablation of endometriotic lesions and lysis of adhesions improves fertility modestly in minimal endometriosis (8, 11, 89, 107, 108). The effect on spontaneous pregnancy favours excision of the endometriotic cysts as compared to drainage and ablation (103).

Artificial reproductive technologies (ART) are beneficial and include controlled ovarian hyperstimulation and intrauterine insemination or IVF and embryo transfer (6).

Ovarian hyperstimulation with clomiphene citrate and insemination improves the chances for pregnancy in women with endometriosis and conservative surgery further improves the outcome, but the treatment is still least successful compared to healthy women (11).

IVF is the most effective treatment for infertility in patients with endometriosis (11). Women with endometriosis are less likely than women with tubal factor infertility to conceive by means of IVF (8) and the success rates after IVF decreases with a more severe disease (11). Long luteal suppression with GnRH agonists for 3-6 months before IVF treatment seem to increase live birth rate compared to shorter down regulation (8, 11, 109). Continuous oral contraceptives for 6-8 weeks seem to be equally effective (16).

There is not sufficient evidence of an effect on reproductive outcome after surgical treatment of endometriomas compared to expectant management before fertility treatment and ovarian surgery may diminish ovarian reserve (6, 8, 16, 103, 110).

#### 1.1.7.3 *Experimental therapies and new approaches*

Large efforts are being made in the development of new therapies for the treatment of endometriosis. The new approaches include modulators of angiogenesis and of the immune system, agents affecting steroid receptors, inhibitors of the local steroid production, GnRH antagonists, statins, antioxidants and agents that inhibit matrix metalloproteinases in the endometriotic tissue (6, 10).

Some immune modulatory drugs have been tried in the treatment of endometriosis.

*Anti-TNF α* (Infliximab®) can inhibit the inflammation process and is used in the treatment of Crohn´s disease and Rheumatoid arthritis (16). A Cochrane review updated in 2012 could not reveal evidence to support the use of anti-TNF α drugs in the management of women with endometriosis for the relief of pelvic pain (111).

*Pentoxifylline* is a xanthine derivative used for arterial sclerosis to improve vascularisation (16). The substance has anti-inflammatory activity and has been tried for the treatment of pain and infertility in patients with endometriosis (10). A systematic review from 2009 based on four trials could not find enough evidence to support the use (112).

*Aromatase inhibitors* (AI) inhibit oestrogen production selectively in endometriotic lesions, without affecting ovarian function (9) and is currently used for breast-cancer treatment. Endometriotic lesions express aromatase and synthesize their own oestradiol and suppression of ovarian oestradiol production may not completely control pain (6, 16). For women in reproductive years AI induce ovulation (8) and should be used together with other hormonal treatments (16). In reproductive-age women, the combination of AI with conventional therapy alleviates endometriosis-related pain and in postmenopausal women, using an AI alone has been shown to be an effective treatment (113). The doses used are lower than those used for breast-cancer treatment and the effect on pain seems to be similar to those of other hormonal therapies (8). Adverse effects include hypo-oestrogenism with hot flushes, joint and muscle pain, headache and negative impact on bone mineral density (16).

*Selective progesterone receptor modulators* aim to correct the progesterone resistance of endometriotic implants (6, 8). Anti progestagens such as mifepristone have been shown to reduce pain in small studies with 9 and 7 patients, but data from large randomised trials are lacking (8). Ulipristal acetate used for pre-surgical treatment of uterine fibroids (Esmya®) and for emergency contraception (ellaOne®) is a selective progesterone receptor modulator under developement for use in endometriosis (8). Asoprisnil, a progesterone receptor ligand and telapristone acetate, a progesterone receptor antagonist are currently studied but results are not yet published (16).

*Oral GnRH antagonists* can induce a dose-dependent decrease in oestrogen levels and are under development (16).

Acupuncture, TNS, hypo gastric nerve block and physical therapy has been proposed but data from large randomised trials are lacking (8).

## 1.2 BACKGROUND LIGNOCAINE

### 1.2.1 Introduction

The first local anaesthetic (LA) substance in clinical use was cocaine, an ester-type local anaesthetic isolated in 1860. Procaine was developed in 1904 and became the dominating LA until 1943 when lignocaine, the first amide-type LA was synthesised. Many LAs have been developed after lignocaine with focus on nerve-blocking effects, duration of action and safety. No ester-type LA have been evolved after 1948 because of larger allergenic potential and since the amino ester-link is more instable (114).
Lignocaine in high concentration has the ability to block sodium channels, thereby blocking membrane excitability and the generation of action potentials (115) and is used for local and regional anaesthesia and for antiarrhythmic treatment. In the last two decades, other beneficial effects of LAs, such as their anti-inflammatory properties, have been investigated.

### 1.2.1 Anti-inflammatory effects of local anaesthetics

Membrane actions of LAs occur not only in excitable membranes but also in non-excitable membranes (115, 116). Inhibition of receptors others than $Na^+$ occur at much lower concentrations, some-times 1000-5000-fold lower (116). In lower concentrations, lignocaine has effects on the inflammatory response and especially on inflammatory cells (116, 117). Most data on anti-inflammatory properties of LA is based on experimental *in vitro* and *in vivo* studies (115).

#### 1.2.1.1 *Anti-inflammatory effects in vitro*

All LAs penetrates the cells and exerts similar therapeutic and anti-inflammatory effects (115, 118). The racemic ropivacaine is the only LA that lacks or has only weak anti-inflammatory properties (115, 119). The substances have effects on several cell functions including collagen synthesis, adhesion to the endothelium, cell motility, NK-cell mediated cell lysis, phagocytosis and the release of inflammatory mediators (119, 120).

LAs induce reversible, conformational and functional alterations of the cell membrane, making it more stable (115, 117, 121, 122). They seem to interact with membrane proteins and lipids, thus interfering with ion channels ($K^+$ and $Ca^{2+}$) and inhibiting the ion exchange (114, 115, 120). Inhibition of intracellular calcium flux may be an important shared mechanism of LAs action on cytokine release and intracellular mechanisms (115, 122, 123). In addition, LAs seem to interact with membrane bound enzyme activity and with the cytoskeleton of the cell (114, 115, 120). The substances can inhibit signalling of G-protein coupled receptors mediating inflammatory response and signalling of muscarinic acetylcholine receptors (116, 124). Effects have also been demonstrated on the cytokine intracellular signalling pathways (122).

In leukocytes, LAs inhibit adhesion to the endothelium, motility, the release of lysosomal enzymes, activation and priming of neutrophils and granulocyte phagocytosis (115, 116, 125). The substances have repressive effects on spontaneous prostaglandin biosynthesis, the release of thromboxane B2, leukotriene and histamine and the production of superoxide anion and hydrogen peroxide (115, 116, 126). Lignocaine can inhibit NK cell activity and suppress eosinophil responses to IL-5 (122, 127).

Lignocaine inhibit macrophage motility (121) and can induce a dose-dependent and reversible inhibition of phagocytosis in macrophages and monocytes (115, 116). In studies of peritoneal macrophages *in vitro*, the presence of lignocaine reduced the phagocytosis of spermatozoa (128). Lignocaine has been shown to decrease cytokine release from macrophages both *in vitro* and *in vivo* (116, 122). It is not known whether the suppressed cytokine release is due to reduced expression and synthesis of the cytokine or decreased cellular secretion (122). However, in one study lignocaine reduce the induced MCP-1 production in human monocytes on both protein and mRNA level and even the cell migration and the peak of cytosolic free $Ca^{2+}$ were dramatically reduced (129). The release of IL-1 by human monocytes was inhibited by lignocaine and bupivacaine (115, 116, 130). Moreover, lignocaine has been shown to inhibit oxidative metabolism in human alveolar macrophages (116).

Lignocaine attenuated the concentrations of endothelial IL-1β, IL-6 and IL-8 in an *in vitro* study with endothelial cells (131). In intestinal epithelial cells, lignocaine inhibited the secretion of IL-8 and IL-1β and also the transcription of IL-8 mRNA was reduced (118). In endothelial and smooth muscle cells lignocaine attenuated cytokine-induced cell-injury and the effect was suggested to be modulated by mitochondrial KATP channels (125).

#### 1.2.1.2 *Anti-inflammatory effects in vivo*

The inflammatory response should protect from tissue damage but an exacerbated inflammatory response rather destroys. The anti-inflammatory effect of lignocaine have been proposed as treatment in various clinical conditions to inhibit tissue damage (123). In animal studies, lignocaine have been beneficial in the treatment of lung injury, septic shock and myocardial infarct (115, 122). Lignocaine accelerates re-epithelialisation and improves wound healing (115). Several studies on rats have shown neuroprotective effects of lignocaine at antiarrhythmic doses (119). In rabbits, lignocaine attenuated the increase in plasma IL-6 and IL-8 concentration induced by intravenously endotoxin administration (132) and inhibited the release of IL-1 and TNF-α in bronchoalveolar fluid from endotoxin-injured lung (118, 133).

In human studies lignocaine have been used as enemas for treatment of ulcerative colitis and the treatment, thought to affect both the nerve cells and the intestinal inflammation, had a high success rate (120). Topical and intravenous lignocaine has been shown to reduce inflammation and pain after burn injuries and topical lignocaine-prilocaine cream in the prodromal stage of herpes simplex could be useful (115). Infusion with lignocaine significantly decreases the incidence of neurological decompression sickness post dive and also shortens the time for recovery of bowel function after surgery (119). Besides, lignocaine has been used for interstitial cystitis (115). In fertility therapy, lignocaine has increased the pregnancy rate when pertubated pre-ovulatory (134, 135). An unexpected side effect was a pain relief in some patients with endometriosis (135). The effect seen on fertility might to some extent be explained by the anti-inflammatory properties of lignocaine but can also be an effect of tubal flushing (136). A small study investigating the effect on pain showed promising results since five of six women with endometriosis reported decreased pain level after pertubation with lignocaine 1,0 mg/ml (137).

The anti-inflammatory effect of local anaesthetics is prolonged and persist after serum levels have decreased (116).

#### 1.2.1.1 *Other effects of local anaesthetics*

LAs in millimolar concentrations possess anti-microbiological properties *in vitro* and *in vivo* (116) but the mechanisms of action for these effects are still unclear (115, 116). All LAs has antimicrobial effects except for Ropivacaine, which has weak antimicrobial properties (115, 119). In addition, the substances have antifungal (115) and antiviral effects in high concentrations (116). LAs also have anti-thrombotic actions (124).

LAs prevent axonal sprouting which might be important in the management of pain together with effects on ion flux and G-protein coupled receptors (114). Both nociceptive and neuropathic pain are targeted by local anaesthetics (114).

### 1.2.2 **Safety**

The adverse effects of lignocaine have been well investigated and manifests most commonly on the central nervous and cardiovascular systems (138, 139). Subjective toxic effects on the central nervous system (CNS) appear at concentrations above 3-5 μg/ml and include nausea, dysphoria, drowsiness and cardiovascular instability (116, 138). Objective adverse manifestations appear at concentrations above 6-10 μg/ml and include disorientation, muscular irritability, respiratory depression, seizures/convulsions and coma (116, 138). Plasma concentrations of lignocaine above 10 μg/ml can affect the cardiovascular system with symptoms such as bradycardia, atrioventricular blockade and cardiac arrest (116). Both hypotensive and hypertensive reactions can occur (116). Serum-levels above 20 μg/ml are required to induce cardiac arrest (138, 140).

Data from the 1960th suggest that large amounts of lignocaine may be infused intravenously before toxicity is produced and the largest dosage in these studies were 200 mg (141). An intravenous (iv) bolus of 2 mg/kg lignocaine results in peak plasma levels of 1,5-1,9 μg/ml and continuous iv infusion of lignocaine 2-4 mg/min leads to plasma concentrations of 1-3 μg/ml after 150 min (116). Serum levels of local anaesthetics after non-vascular administration correspond with the vascularity of the tissue (114, 140). Rectal application of 400 mg lignocaine resulted in maximal plasma concentration 0,5-1,9 μg/ml after 2 h (120). Small molecules like lignocaine hydrochloride with a molecular weight of 271 Da diffuse rapidly through the peritoneum (28, 31). In a review of systemic levels of LAs after intraperitoneal application, nine trials where lignocaine were used were found (139). The dosage used varied from 100 mg-1000 mg and the time to maximum concentration ($T_{max}$) in serum ranged from 5 to 40 minutes for plain lignocaine. Mean concentration maximum ($C_{max}$) ranged from 1.01 μg/ml to 4.32 μg/ml and the highest observed value was detected after intra peritoneal administration of 400 mg lignocaine (142). No report of serum or clinical toxicity was found in any of the reviewed studies (139).

Tissue toxicity, primary myotoxicity and neurotoxicity can be produced by all LAs if "high" concentrations are used but true allergic reactions are associated only with amino ester-linked LAs (114).

The anti-inflammatory properties of LAs might in theory increase the susceptibility to infections but this has not been relevant in the *in vivo* studies reported to date except in settings of gross bacterial contamination (116). LAs seem to be able to modulate excessive inflammation without significant impairment of host defences (116) and no increased infection rate attributable to an anti-immune effect of LAs has been demonstrated (122). LAs selectively inhibit the priming of polymorphonuclear cells and prevent overactive inflammatory responses without impairing host defence or suppressing normal inflammation (119). Patients treated with lignocaine are not more susceptible to infections (119).

Lignocaine passes through the placenta (143) and human foetus and newborn have the ability to metabolise lignocaine to the same extent as adults (143). No embryotoxic effects of lignocaine have been found in rats (143) but no studies regarding embryo toxic effects of lignocaine in humans have been conducted. Lignocaine has been used in numerous women during pregnancy and there is no evidence supporting that lignocaine could disturb the reproduction process according to FASS (the Swedish Pharmacopeia Drug Information) and JanusInfo (Interface Management of Pharmacotherapy).

## 1.3 PATIENT REPORTED OUTCOMES

### 1.3.1 Definition

Patient reported outcomes (PRO) are measurements based on a report that comes directly from the patient about their health condition. PRO includes Quality of life (QOL) which has been established as an important end-point in clinical trials. QOL is a multidimensional and subjective concept including physical, social, and psychological functioning. Health status, functional status and QOL are concepts often used interchangeably to refer to the same domain of health. However, patients distinguish between QOL and health status which are perceived as two distinct constructs (144). When rating QOL patients give greater emphasis to mental health than to physical functioning and the pattern is reversed for appraisals of health status (144). Health related quality of life (HRQL) is often used and encompasses physical, emotional and social aspects associated with a disease or its treatment (145, 146).

### 1.3.2 Quality of life questionnaires

There are several questionnaires for evaluating HRQL and the questionnaires could be generic or disease specific. Generic instruments are intended for general use and enables comparison across disease groups. Disease-specific questionnaire are meant for use in a specific disease area and are more sensitive and responsive to change than the generic instruments (146, 147). For endometriosis, three disease specific questionnaires have been developed by Bodner 1997 (148), Colwell 1998 (145) and Jones 2001(146).
For use in clinical trials a questionnaire should be valid, reliable, sensitive and responsive to change (149, 150). Validity means that the questionnaires measure what they are intended, and appear, to measure (151). Reliability means that the scale yields reproducible and consistent results and can discriminate between better and worse HRQL within and between individuals (151). Internal reliability measures homogeneity of items within a scale or the precision of a scale (152). Sensitivity is the ability to detect clinically relevant differences between groups, for example between two treatment groups in a randomised clinical trial and

is evaluated indirect through data quality (147). Responsiveness measures how sensitive the questionnaire is to detect and describe changes in patients' health status over time (153). Responsiveness refers to the instruments ability to detect small but important changes with feasible sample sizes and can be seen as an aspect of construct validity (150).

### 1.3.3 Evaluation of responsiveness

Responsiveness must be demonstrated and documented for the particular study population and it is highly recommended to confirm responsiveness across multiple samples (150). The responsiveness is evaluated through data quality by longitudinal assessment of patients in whom a change is expected to occur (147, 150). Some criteria or "anchors" are needed to identify whether patients have changed over time and should have relationship with the PRO measure (150). Responsiveness can be determined by comparing the questionnaire to a generic health status questionnaire with established responsiveness or by using patient-rated global improvement i.e. transition questions (153). A transition question requires the respondent to evaluate the change in their clinical status or their health status by answering the response categories much better, somewhat better, about the same, somewhat worse and much worse (150, 153).

Two of the most widely used measures of responsiveness are the Effect size (ES) and the Standardised response means (SRM) (147, 150). Also the Index of responsiveness can be used (145, 150, 153). There is controversy as to which is the best measure to use for evaluating responsiveness and the preferred index is unclear (147). All these statistics is the observed amount of changes over the observed amount of variation (152).

*Effect size (ES)* is one of the most commonly used methods for interpreting change in a score and is an estimation of the magnitude of change in health status between two different times (154, 155). ES is independent of sample size and the standardized mean difference (*d*) calculated as Cohen *d* is the standard measurement for effect size. Cohen *d* is calculated by dividing mean difference with the pooled standard deviation (SD) (147, 156). The pooled SD represents the root mean square of the two standard deviations. An effect size of 0.2 indicates small change, 0.5 indicates a moderate change and 0.8 a large change as proposed by Cohen *et al.* (156). The ES index gives information about the size of an effect in terms of SD units. An ES of 0.0 indicates that the mean of the treated group is at the $50^{th}$ percentile of the untreated group and an ES of 0.8 indicates that the mean of the treated group is at the $79^{th}$ percentile of the untreated group (156).

*The standardised response mean (SRM)* is the ratio of the mean changes to the SD of that change i.e. mean change on a scale divided with the mean change in SD (147, 153). An SRM >0.5 is considered to be responsive (151).

*Index of responsiveness* is the ratio of the average change score in those with improvement (or deterioration) to the SD of the change in the stable group (145, 151). An index of responsiveness greater than 1.0 is considered indicative of a measure highly responsive to change, whereas a value of 0.2 or more is considered acceptable (145, 153, 157).

To evaluate responsiveness, one can also compare differences in changes in the scores across different groups (i.e. stable versus moderate improvement) (150, 158). For evaluating the significance of change scores a paired t-test can be used (153, 158).

All of the above methods are based upon means and SD, with an implicit assumption that the data follow a normal distribution. Many QOL scales have a non-normal distribution, in which case medians and interquartile ranges (IQR) may replace means and SD (147, 159). Unfortunately little work has been carried out into this subject (147). Moreover, it should be noted that some scales are not only non-normal, but may also suffer from "ceiling effects" in which a large number of patients place responses in the maximum category (147). This can compromise sensitivity and responsiveness (147, 151).

### 1.3.4 Evaluation of data quality

Floor and ceiling effects refer to the extent to which patients score at the extreme ends of the questionnaire and are considered to be present if >15% of respondents achieved the lowest (floor) or highest score (ceiling) (160, 161). If all patients score at extreme ends, it is not possible to detect an improvement or deterioration. The data quality also consists of evaluation of missing items in percent and gives an indication of whether the questionnaire is acceptable and understandable to the respondents (161).

### 1.3.5 Questionnaires to be used in endometriosis

Outcome measures for use in international clinical trials in endometriosis with regard to pain symptoms were established on an international meeting convened by the National Institutes of Health (NIH) and ASRM. Ratings on an 11-point NRS (Numerical/numeric Rating Scale) such as the VAS-scale, was recommended as primary outcome measures (74). For secondary outcome measure they proposed the Biberoglou and Behrman scale (categorical, 0-3) and the Endometriosis Health Profile-30 (EHP-30) questionnaire (74). A definition of responders was suggested to be either a 30% or a 50% reduction in symptoms (74).

#### 1.3.5.1 *The VAS-scale*

The visual analogue scale (VAS) scale is ranging from 0 i.e.no pain to 100 corresponding to the worst pain imagined. The VAS scale is a valid instrument for evaluating chronic pain during endometriosis (74, 162). It is easy to administer and score, is sensitive to treatment effects and correlates with other intensity measures (74).
There are few studies defining the clinical relevant improvement on the VAS scale. In 2010 Gerlinger et al. tried to define a minimal clinically important difference for endometriosis-associated pelvic pain. In that study, based on two small but randomised controlled trials, they found that the best separation between women rating themselves "minimally improved" and "improved" was a decrease of 28 mm on the VAS scale (163). The VAS scale is considered to be an ordinal scale and non-parametric statistics should therefore be used.

#### 1.3.5.2 *The Biberoglou and Behrman scale*

The Biberoglou and Behrman scale from 1981 (164) has been widely used in clinical studies but have considerably limitations. It asks the patients about function and is not a pain scale. The scale include three symptoms; dysmenorrhea, dyspareunia and chronic pelvic pain,

graded on a scale from 0-3. Also, pelvic tenderness and induration at examination is evaluated by the physician on a scale from 0-3. A standard is lacking for the symptoms that will indicate that a treatment has succeeded (74). The Biberoglou and Behrman scale has not been used in a consistent manner and has never been validated or shown to be reproducible (74).

#### 1.3.5.3 *The EHP-30*

The Endometriosis Health Profile-30 (EHP-30) questionnaire is the only QOL scale that has been validated for use in women with endometriosis (74). The questions in the EHP-30 questionnaire were patient-generated and the questionnaire has proved to be reliable, valid and responsive to change (146, 153, 161). When the responsiveness of the EHP-30 was initially evaluated, complete data were obtained for 40 patients, and the study showed that only the social support scale on the core questionnaire failed to demonstrate any responsiveness (153). Responsiveness has thereafter been demonstrated for all scales in a larger sample of 228 women in the Netherlands (165). The EHP-30 questionnaire has been shown to be more responsive to change compared to the generic tool Short Form-36 (SF-36) in patients with endometriosis (153, 166).

The EHP-30 questionnaire comprises two parts: a core questionnaire, which consists of five scales (pain, control and powerlessness, emotional well-being, social support and self-image) with a total of 30 items applicable to all women with endometriosis. The other part is the modular questionnaire, which does not necessarily apply to all women with endometriosis. It consists of six scales (work life, relationship with children, sexual intercourse, infertility, medical profession and treatment) and contains a total of 23 items (167). Within the scales the items are summed to create a raw score and each scale is then translated into a score ranging from 0 (best health status) to 100 (worst health status). All scores should be presented separately (168). The EHP-30 questionnaire is acceptable and understandable to the respondents indicated by high rate of data completeness and has low floor and ceiling effects (161).

### 1.3.6 **How to interpret the results from PRO**

To be able to interpret results from a clinical trial including PRO and HRQL, the questionnaire have to be valid, reliable, sensitive and responsive to change (149). The questionnaire also needs to be acceptable and understandable to the respondents and have interpretation guidelines, for example minimal important difference (MID) (150).

The Minimal important differences (MID) can be defined as the smallest difference in score that the patients perceive as a change and can be used to interpret HRQL outcomes in clinical trials (169, 170). MID is the subjective significant change and a threshold that demarcates trivial from small but important differences (171). The MID may vary by population and context and no one MID will be valid for all study applications involving a PRO instrument (150, 171). Both responsiveness and MID must be demonstrated and documented for the particular study population (150). The MID of a HRQL instrument can be estimated using within-patient global ratings of change (170).

The effect of any treatment should be expressed in terms of both statistical significance and clinical relevance. Statistical significance testing is depending on both the size of the effect

and the size of the sample and is the golden standard for interpreting results in clinical trials. However, a significant change does not mean that the change is clinically important and can be perceived by the patients. Whether or not an effect is clinically meaningful should be determined by the patients (74). Effects of an intervention on health status should ideally be analysed in two ways; as mean differences between patient groups in the change in scores and as the difference in the proportion of patients in both groups exhibiting clinically significant change as defined by the MID (151, 169). Even if the mean difference between treatment and control group is less than the MID, treatment may have an important impact on many patients (172). Investigators are recommended to report the estimated threshold and either a change in score or an absolute score obtained can be referred to (169, 170).

Using effect sizes can be a way to interpret changes in health status and can assess the magnitude and meaning of health status changes (159). Common types of effects sizes are odds ratio and risk ratio. The standardized mean effect (or Cohen *d*) is another type of effect size and expresses the mean differences between two groups in standard deviation units. Effect sizes are mostly used for comparing and integrating results across studies, as in meta-analyses and for performing power analyses (159).

### 1.3.7 The placebo effect

Despite the assumption that placebo treatment is ineffective, there is a wealth of information to the contrary. An increased endorphin production in association with placebo analgesia has been described (173, 174). Placebo effects in the range of 40-45 % improvement in symptoms have been reported in studies monitoring pain (8, 175) and the average strength of placebos upon pain on a VAS scale is 2 out of 10 units (176, 177). When patients in a clinical trial were treated with "warmth, attention and confidence" the response to a placebo increased from 44 % to 62 % (178). Larger effects of placebo interventions are associated with physical placebo interventions (like sham acupuncture), patient involved outcomes, small trials and trial with the explicit purpose to study placebo (179). In a Cochrane review, the placebo effect could be documented only in studies in which the subjects themselves reported the outcomes and the improvements in pain were small and could not be clearly distinguished from reporting bias (179). Response bias or reporting bias is the tendency for patients to report their symptoms in a way they feel are socially acceptable or desirable or the way they think the doctor wants (180). In another meta-analysis, placebo had a significant effect on pain when measured on continuous scales whereas no effect was seen when the outcome was binary (181).
Thus, placebo interventions in general do not have any clinically important effects but can have possible small benefits in studies with continuous subjective and patient-reported outcomes and for the treatment of pain (179). In certain settings, placebo interventions can influence patient-reported outcomes, especially pain and nausea, though it is difficult to distinguish patient reported effect of placebo from biased reporting (179).

# 2 OBJECTIVES

Previous studies have indicated that pertubation with lignocaine could relieve pain in patients with endometriosis (135, 137). This effect was unexpected and was spontaneously reported by the patients who did not achieve pregnancy in a fertility study in 2001 (135). The effect on pain was further evaluated in a small but randomised study in which different concentrations of lignocaine was evaluated. Five of six patients who had been treated with the highest concentration of lignocaine (1mg/ml) reported reduced dysmenorrhea (137).

The purpose of all the present studies was to further evaluate the effect of pertubation with Ringer-Lignocaine in women with endometriosis and to find an explanation for a possible effect.

A double-blind, randomised controlled trial was carried out to evaluate the effect of pertubation with lignocaine 1mg/ml on dysmenorrhea and quality of life in patients with endometriosis. The hypothesis was that pertubation with lignocaine could relieve pain and improve quality of life in patients with endometriosis.

The safety of the method and the pharmacokinetics of lignocaine after pertubation needed to be evaluated. Our hypothesis was that the pertubated dosage of 10 mg lignocaine hydrochloride reaches the central circulation and gives rise to low systemic levels. Pertubation with lignocaine was thought to be safe and without side effects.

The social support scale on the EHP-30 core questionnaire had previously failed to demonstrate responsiveness. The responsiveness should be confirmed in the particular study population and the question was raised whether our results from the EHP-30 questionnaire were reliable. The primary objective of the fourth study was thus to evaluate the responsiveness and the applicability of the EHP-30 questionnaire in our Swedish sample. The hypothesis was that the EHP-30 questionnaire was applicable and responsive for change for all dimensions on the EHP-30 core questionnaire.

The cytokines in the peritoneal fluid might play a part in the symptoms of endometriosis. Lignocaine has anti-inflammatory properties and can decrease cytokine release both *in vitro* and *in vivo*. With the aim to find an explanation for the clinical effects seen *in vivo,* the effect of lignocaine on cytokine expression and secretion from peritoneal fluid macrophages and ectopic endometriotic stromal cells was evaluated *in vitro.* It was hypothesized that lignocaine could attenuate the expression and release of the pro-inflammatory cytokines IL-6, IL-8 and MCP-1 in cell cultures.

# 3 PARTICIPANTS

## 3.1 ETHICS

The studies I-IV were approved by the Medical Products Agency in Sweden, Nov. 8, 2006 (Dnr 151:2006/56028) and after amendment Dec 12, 2007 (Dnr151:2007/76934) as well as by the Regional Ethical Review Board in Stockholm, Jan 10, 2007 (Dnr 2006/1416-32) and after amendment Dec 14, 2007 (Dnr 2007/1398-32). Study V was approved by Regional Ethical Review Board in Stockholm, 2007-11-21 (Dnr: 2007/988).

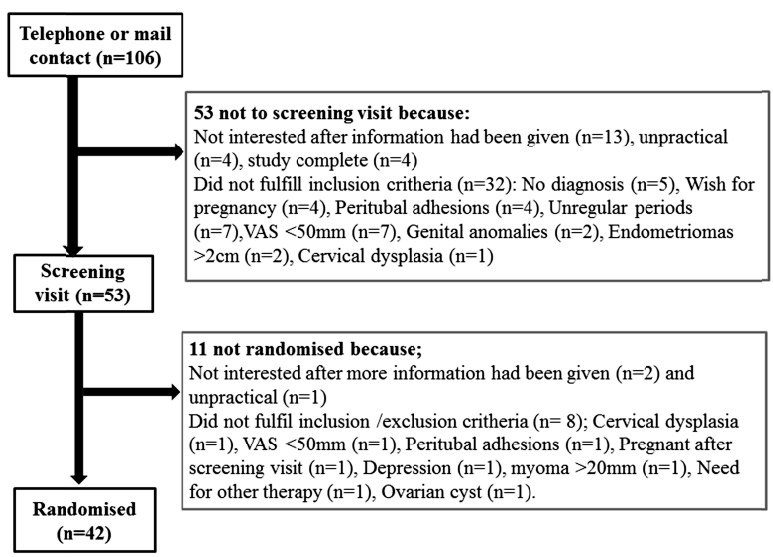
## 3.2 SUBJECTS AND SETTING

In total 59 women have participated in the studies of which 53 had endometriosis and six were healthy controls. Treatments were given at three sites in Stockholm, Sweden. Written informed consent was obtained before any study related procedures and the CONSORT (Consolidated Standards of Reporting Trials) guidelines were followed.

### 3.2.1 Enrolment

Women in study I-IV were recruited through advertisements and from the gynaecological outpatient unit at the participating clinics. The participating clinics were Danderyd hospital (n=35), Läkargruppen Victoria (n=3) and Karolinska University Hospital in Huddinge (n=4). Initially, 106 women with dysmenorrhea due to endometriosis expressed interest (by mail or phone) in participating in the study. 53 patients came for screening visit and 42 were randomised, 24 to lignocaine and 18 to placebo. Reasons for not participating included failed interest after information had been given or not fulfilling the inclusion criteria (figure 1).

**Figure 1; Enrolment study I-IV**



The women in study V were recruited at the Department of Gynaecology, Danderyd Hospital. Women that were scheduled for surgery for clinical reasons (endometriotic cyst enucleation, n=10, laparoscopic sterilisation, n=7) were asked to participate. Information was given at the screening visit the day before surgery and all women gave their informed consent.
Samples from 15 women were collected. One patient undergoing laparoscopic sterilisation was found to have peritoneal endometriosis. Two of the women with endometriosis never got operated and samples were obtained from nine women with endometriosis and six healthy controls. One sample from a woman with endometriosis and one from control were used for testing the cell culture protocol.

### 3.2.2 Inclusion and exclusion criteria

The main inclusion criteria in study I-IV were presence of peritoneal or ovarian endometriosis as verified by laparoscopy and dysmenorrhea with a pain score of >50 mm on the VAS scale. Main exclusion criteria were reduced patency in the Fallopian tubes and intention to achieve pregnancy during the forthcoming year. Detailed eligibility criteria are presented in table 1.

**Table 1; Inclusion and exclusion criteria study I-IV**

| Inclusion criteria | Exclusion criteria |
|---|---|
| • age > 20 years<br>• endometriosis verified by laparoscopy<br>• dysmenorrhea or pelvic pain defined as a pain score of >50 mm (VAS)<br>• normal Fallopian tubes<br>• regular menstrual cycles 21-35 days<br>• treatment with oral contraceptives (OC) ongoing >1 month and continued during the trial<br>• previous hormonal treatment discontinued > 1 month (OC, gestagens) and > 6 months (GnRH agonist)<br>• no wish for pregnancy during the study<br>• normal Pap smear,<br>• negative chlamydia test<br>• negative pregnancy test<br>• informed consent given and signed | • continuous treatment with medication that may increase risk for infection<br>• clinical signs of pelvic inflammatory disease<br>• hyper reactivity to local anaesthesia<br>• fibroids > 2 cm<br>• ongoing treatment with GnRH agonist<br>• ongoing continuous treatment with high-dose gestagens<br>• pregnancy<br>• peritubal adhesions<br>• occluded Fallopian tubes<br>• inability to understand information or comply with study procedures<br>• participation in a clinical study within one year before the present study.<br>• any disease or laboratory finding considered of importance by the investigator for not including the patient |

To be included in study V, the women should be 18 to 45 years of age and either fertile and without endometriosis or have visible endometriosis at surgery. The included women should also understand written and spoken Swedish. Exclusion criteria were ongoing treatment with GnRH or gestagens or on-going pregnancy.

### 3.2.3 **Demographics**

The medical history and demographic characteristics of the patients in lignocaine and placebo groups were comparable (table 2).

**Table 2; Demographics and medical history study I-IV**

| | **Study I, III and IV** | | | | **Study II** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Placebo (n=18)** | | **Lignocaine (n=24)** | | **Placebo n=9** | | **Lignocaine n=16** | |
| **Parameter** | **Mean (SD)** | **Min-max** | **Mean (SD)** | **Min-max** | **Mean (SD)** | **Min-max** | **Mean (SD)** | **Min-max** |
| Age years | 33.4 (4.4) | 26-39 | 33.08 (5.5) | 22-43 | 32.7 (5.6) | 26-40 | 34.1 (5.8) | 25-44 |
| Weight kg | 67.6 (12.2) | 50-98 | 69.5 (11.1) | 50-90 | 69.8 (15.3) | 50-98 | 66.9 (11.2) | 50-90 |
| Height cm | 167.4 (8.6) | 156-181 | 164.0 (4.6) | 154-172 | 168.3 (9.9) | 156-181 | 164.3 (4.5) | 155-172 |
| Duration endometriosis[1] | 4.5 (4.46) | 0-16 | 5.5 (4.04) | 0-13 | 3.9 (3.9) | 0-12 | 6.0 (3.8) | 1-11 |
| VAS at inclusion | 78.22 (18.62) | 43-100 | 73.58 (19.0) | 17-99 | | | | |
| Diastolic BP at inclusion, | 74 (7.9) | 60-92 | 77 (9.8) | 63-104 | 76.0 (8.8) | 67-92 | 76.8 (8.5) | 63-90 |
| Systolic BP at inclusion | 118 (13.0) | 90-148 | 121 (12.2) | 105-156 | 118.4 (17.9 | 100-148 | 121 (96) | 105-140 |
| Heart rate | 68.4 (7.2) | 54-80 | 73.0 (10.0) | 58-96 | 67.3 (5.9) | 60-76 | 72.1 (9.4) | 58-91 |
| | **Number** | | **Number** | | **Number** | | **Number** | |
| Smokers | 0 | | 4 | | 0 | | 2 | |
| Patients using SSRI | 4 | | 3 | | 2 | | 2 | |
| Patients using analgesics | 18 | | 24 | | 9 | | 16 | |
| Paracetamol | 12 | | 14 | | 2 | | 9 | |
| NSAIDs | 13 | | 22 | | 6 | | 19 | |
| Codeine | 6 | | 5 | | 4 | | 4 | |
| Tramadol | 1 | | 2 | | 1 | | 0 | |
| Dextropropoxyphene | 1 | | 4 | | 0 | | 2 | |
| Other opioids | 2 | | 3 | | 2 | | 0 | |
| Oral contraceptives | 3 | | 2 | | 3 | | 2 | |
| Intrauterine device | 0 | | 1 | | 0 | | 1 | |
| Levothyroxine | 2 | | 1 | | 2 | | 1 | |
| Corpus luteum cyst | 3 | | 1 | | 2 | | 1 | |
| Endometrioma | 0 | | 2 | | 0 | | 1 | |

1. Years since diagnostic laparoscopy

A total of 17 patients were included in study V, of which eleven had endometriosis and six were healthy controls. One patient undergoing laparoscopic sterilisation was found to have peritoneal endometriosis and was therefore considered to have endometriosis. The mean age was 34.7 years for women with endometriosis and 41.1 years for the healthy controls. Two women were on contraceptive pills (one healthy, one endometriosis), one had been treated with progestin 16 days earlier (healthy) and one had a hormonal intrauterine device (endometriosis). Two women were treated with SSRI (both healthy).

# 4 METHODS

## 4.1 STUDY DESIGN

Study I and III were both randomised, double-blinded and controlled clinical studies. The effect of pertubation with lignocaine on pain (study I) and quality of life (study III) was evaluated. The patients were sequentially randomised to receive study treatment (pertubation with lignocaine 1mg/ml in Ringer solution) or placebo (pertubation with Ringer solution). Three treatments were to be given pre-ovulatory on cycle day 6-12 in three sequential menstrual cycles. The effect was evaluated with two questionnaires concerning pain and health related quality of life.

Study II and IV were both prospective observational studies. In study II the concentration of lignocaine in serum after pertubation was measured and presented. In study IV the responsiveness of the EHP-30 questionnaire was evaluated.

Study V was an experimental *in vitro* study on human cells.

## 4.2 POWER CALCULATION

The primary outcome in the randomised study was the effect on pain and a power analysis was conducted for this outcome. The power calculation was based on a preceding study in which five of six patients were improved after pertubation with lignocaine 1.0 mg/ml (137). In the power analysis using the Chi square test at 80 % power, it was assumed that 60% of the subjects given the study treatment and 20% in the control group would improve. To achieve a statistical significance ($p<0.05$, using Fisher's exact two-sided test), 20 subjects had to be randomised in the treatment group and 15 subjects in the control group.
A 4:3 treatment/placebo rate was used to gain more safety data and to encourage women to participate since the chance of active treatment compared to placebo was higher.
A power calculation was not made for the secondary outcome quality of life since no preliminary results for the effect on quality of life were available.

## 4.3 PROCEDURES (STUDY I-IV)

Patients included in study I-IV had four visits of which the first was a screening visit and the other three were treatment visits. The treatments were given over three sequential menstrual cycles. Data from a previous study had given indications that the effect on dysmenorrhea increased after repeated treatments (135), hence it was decided to use three treatments.

### 4.3.1 First visit

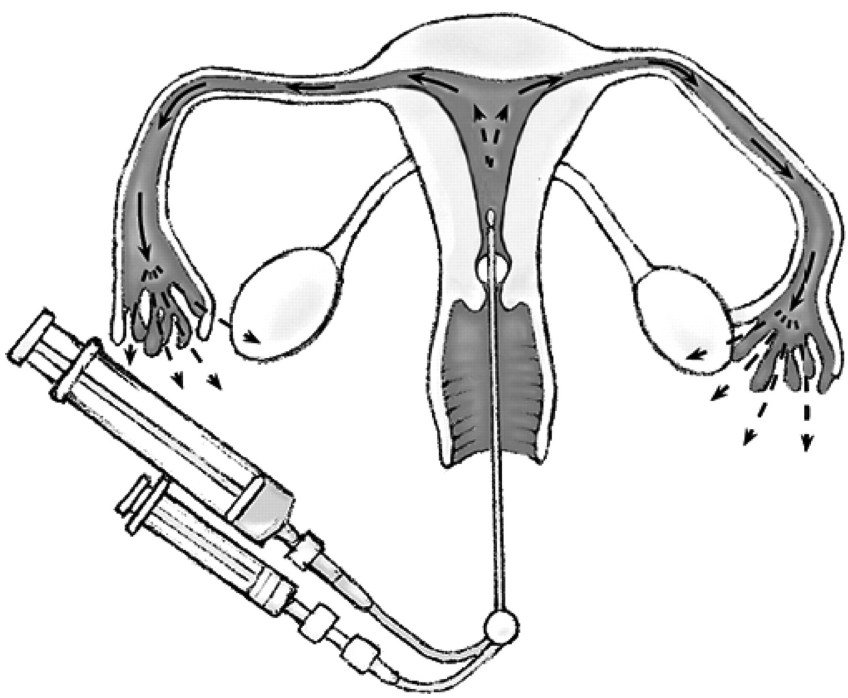
At the first visit, the study patients were scrutinised concerning the inclusion and exclusion criteria. A physical examination including blood pressure, gynaecological examination including wet smear and a vaginal ultrasound were performed. Chlamydia and pregnancy tests were taken and bacterial vaginosis, if present, was treated. The subjects should have had a normal Pap smear taken within the previous three years and if not a new smear was carried out. Demographic data, concomitant medication and medical history were recorded. The patency was considered normal if the preceding laparoscopy revealed normal Fallopian tubes anatomy. In five patients, three in the placebo group and two in the lignocaine group,

information concerning patency of the Fallopian tubes was missing in the patient records or history, and therefore hystero salpingo contrast sonography was performed prior to study entry.

### 4.3.2 Second, third and fourth visit

At the second visit, the patients were randomised sequentially as they were eligible. At the treatment visits, any changes in concomitant medication, medical status or presence of any adverse events (AE) since the preceding visit were recorded. Before each treatment a pregnancy test and a gynaecological examination including wet smear were performed to confirm the absence of bacterial vaginosis or signs of pelvic inflammatory disease. The pertubations were carried out on menstrual cycle day 6-12 since some patients were in natural cycles and could possibly get pregnant. A thin plastic catheter (PBN-Medicals, Stenløse, Denmark) was inserted in the cervical canal or in the distal part of the uterine cavity and a small intraluminal rubber balloon on the catheter was inflated with saline to prevent retrograde leakage. Blood pressure and heart rate were measured and recorded before and five minutes after the treatment. 10 ml of solution was infused through the uterine cavity and pertubated into the peritoneal cavity (figure 2). The solution was infused with vaginal ultrasound supervision during approximately 5 minutes and the fluid could be seen in the pouch of Douglas after treatment. The patients did not receive lignocaine by any other route during the study.

**Figure 2; The pertubation process**



### 4.3.3 Randomisation

The patients were randomised sequentially as they were eligible. Solutions for pertubation were produced and released in a double-blinded manner (APL/Apoteket Production & Laboratories, Box 6124, SE 906 04 Umeå, Sweden). The double-blinded study solutions were delivered to the sites in blocks of treatments for seven patients: three placebo and four study treatment kits. In total six blocks were completely used. After randomisation of a patient, each site assigned the patient to the next available treatment kit in the on-going block. The randomisation list remained at APL until a clean file was declared.

### 4.3.4 Serum sampling (study II)

At one occasion, serum samples were collected and for practical reasons at only one of the study centres (Danderyd hospital). All patients that accepted the serum sampling at this centre were included in study II (n=25). A peripheral venous catheter was inserted in vena brachialis before the treatment and a 10 ml blood sample was collected at 0, 5, 15 and 30 minutes after pertubation i.e. totally 40 ml. The samples were centrifuged, the serum was stored at –70 degrees Celsius and later analysed in one batch for the concentration of lignocaine. The samples were collected from April 2007 until Nov 2008 and the analyses were made in April 2009. Since the study was blinded, tests were taken on both patients who received lignocaine (n=16) as well as placebo (n=9).

The concentration of lignocaine in serum was determined with a LCMS-SIM method (OncoTargeting AB. Rapsgatan 7, 754 50 UPPSALA). The smallest observed peak with this method was 6 nM (1.4 ng/ml), the detection limit was 18 nM (4.2 ng/ml) and the limit of quantification was 60 nM (14.1 ng/ml).

### 4.3.5 Per protocol and Intention to Treat (study I and III)

The intention to treat (ITT) population consists of all women randomised. Previous studies had given indications that the effect on dysmenorrhea increased after repeated treatments (135) and in the present study three pertubations during a maximum of five menstrual cycles was considered a successful treatment, thus fulfilling the prerequisites to be included in the Per Protocol (PP) population.

## 4.4 FOLLOW UP AND QUESTIONNAIRES

### 4.4.1 Pain and dysmenorrhea (I, III and IV)

The effect on dysmenorrhea in study I was evaluated with a pain questionnaire including a VAS scale, initially filled out at the menstruation before the first treatment, i.e. baseline. Thereafter the pain questionnaires were completed during the 2nd, 3rd and 4th period, i.e. after every treatment. The final follow-up took place after the 7th, 10th and 13th menstrual period, i.e. 6, 9 and 12 months after initial treatment. The maximum pain on the VAS scale during every menstrual period was recorded and a decrease on the VAS scale of ≥50% from baseline was defined as a success. Initially, also a decrease on the VAS scale of >40 mm from baseline was defined as a success. This outcome was however deleted from the published version due to comments from reviewers. Obtaining a VAS <20mm was also

evaluated and considered a secondary end point since it indicate a low pain level. The change on the VAS scale was the primary outcome in the first study.

The pain questionnaire used was a revised version of the Biberoglu and Behrman scale (1981) including the three symptoms; dysmenorrhea, dyspareunia and chronic pelvic pain. The function because of pain was evaluated by patients on the categorical scale (0-3), ranging from no pain to bedridden for the major part of the day. The dysmenorrhea was evaluated on menstrual cycle day 1, 3 and 5. The sum of the pain scores from the categorical scales (0-12) were compared between the different time points and a decrease by ≥2 from baseline was defined as a treatment success.
The participating patients in study I were also asked to report the use of rescue medication, their need to be on sick leave and to estimate any changes in their overall pain level, all of which were secondary outcomes.

The time point for primary efficacy evaluation was after the third and last pertubation that corresponded to the fourth menstruation approximately three months after the initial treatment. The secondary time points for primary efficacy evaluation were after 6, 9, and 12 months respectively.

In addition, the patients were asked to estimate changes in their overall pain level during and between periods by answering the response categories “much better”, “somewhat better”, “about the same”, “somewhat worse” or “much worse”. This corresponds to the global question on the general quality of life questionnaire SF-36 (appendix 9.4) and can be used to examine the responsiveness of an instrument (166) and to calculate the minimal important difference (170).

The estimated change in pain intensity and the results from the EHP-30 questionnaires were used to evaluate the responsiveness of the EHP-30 questionnaire in study IV. The patients in the lignocaine and the placebo groups were in study IV analysed all together and were grouped according to their own estimation of change in pain intensity. The patients that estimated their pain to be “somewhat better” during and/or between periods were classified as better (n=17) and the patients that felt “somewhat worse” or “much worse” during and/or between periods were classified as worse (n=8). Patients that estimated their pain to be “about the same” both during and between periods were classified as “same” (n=6) and the two patients that became “much better” both during and between periods were classified as pain free. One patient was removed from analysis since she could not be classified according to the above definition. She became pain free between periods whereas the pain during periods became worse.

The patient´s estimation of change in pain intensity was also used in study IV and III to calculate the minimal important differences (MID) on the EHP-30 questionnaire. The MID for the scores on the EHP-30 in our material correlate to the mean change from baseline to follow up after six months for patients who felt “somewhat better” considering pain intensity after six months and excluding the ones that became pain-free (Appendix 9.1. Pain questionnaire).

### 4.4.2 **Quality of life (study III and IV)**

The effect on QOL was evaluated with a Swedish translation of the EHP-30 questionnaire (Pharmacia UpJohn, 2001) and were initially filled out before the first treatment i.e. baseline. The follow-up took place after the 7th and 13th menstrual period, i.e. six and 12 months after initial treatment. The patients received the treatments before the 4th period and no treatments were given the subsequent two periods preceding the collection of the EHP-30 questionnaire after six months. All dimensions and items on the core questionnaire were collected. On the modular questionnaire the score concerning sexual intercourse (five items) were included.

If one or more items were missing from any dimension on the core and modular questionnaire, a scale score could not be calculated for that individual (168). Only the complete scores are presented for the different dimensions giving different number of patients in various dimensions. Furthermore, if any item was missing in any dimension at baseline, this specific score was withdrawn from further analysis concerning this specific dimension. All scores on the different dimensions on the EHP-30 are ranging from 0 (best health status) to 100 (worst health status). A decrease on a score scale (i.e. negative change) at follow up implies that the patient has improved considering this dimension on quality of life (Appendix 9.2.).

## 4.5 **PROCEDURES STUDY V**

### 4.5.1 **Study material**

#### 4.5.1.1 *Endometriotic cyst capsules (ECC)*

From patients with endometriosis (n=7), a part of the endometriotic cyst capsule (ECC) was obtained after enucleation. Five ECC were collected from women in the proliferative phase and two in the secretory phase of the menstrual cycle.

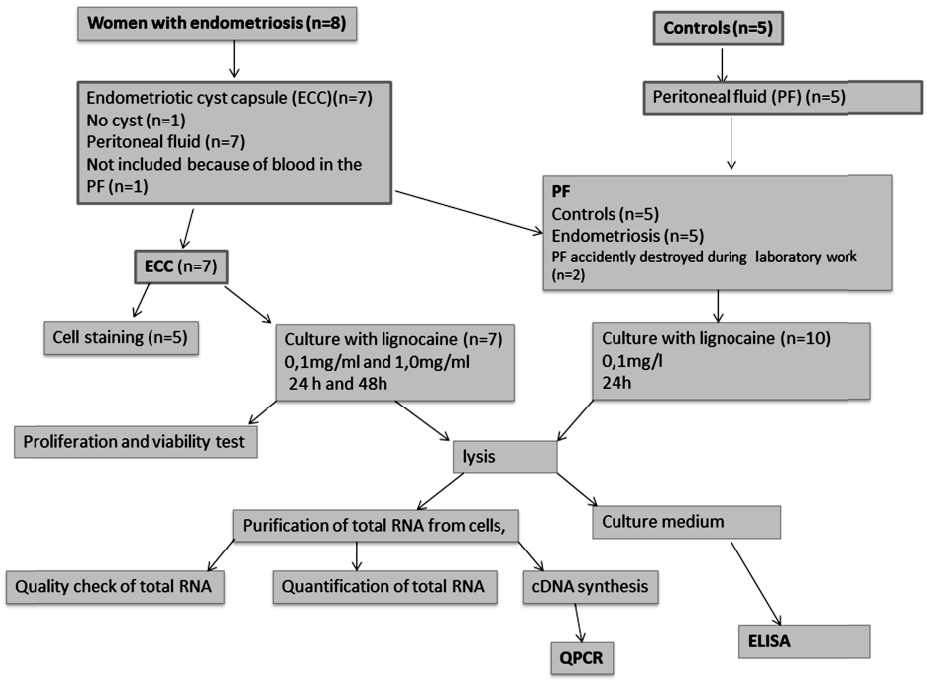
#### 4.5.1.2 *Peritoneal fluid (PF)*

For isolation of monocytes/macrophages, peritoneal fluid (PF) was collected from all participating women. The peritoneal fluids from the patients with endometriosis were obtained in the secretory phase (n=2) and in proliferative phase (n=3), respectively. The peritoneal fluids from controls were collected during the secretory phase (n=3), the proliferative phase (n=1) and one from an amenorrhoic woman who used oral contraceptives.

### 4.5.2 Cell cultures and treatment with lignocaine

A flow chart of the *in vitro* experiments can be seen in figure 3.

**Figure 3; Flow chart of *in vitro* experiments**




#### 4.5.2.1 *Cells from the endometriotic cyst capsule*

Samples from the cyst capsules were immediately put in Hanks buffer (Life technologies), kept at room temperature and transported to the laboratory at Uppsala University Hospital within 3 hours. The cells covering the inside of the endometriotic cysts were detached from the fibrous outer layer by gentle scraping with a scalpel and thereafter put in RPMI1640 and centrifuged at 1500 x g for 2 min. The cells were then treated with collagenase II (2.5µg /ml in PBS, Sigma), and incubated in 5% $CO_2$ at 37°C for 2h. Thereafter the cells were centrifuged at 400 x g for 5 min and then re-suspended in RPMI 1640 medium containing 20% foetal calf serum, HEPES buffer (0.015M) , Sodium-Pyruvate (1mM), MEM Nonessential amino acids (1:100), Penicillin-Streptomycin (1:50) ECGS 0.5µg/ml, Heparin 0.90µg/ml (0,250 ml in 25 ml) and L-glutamine (4mM).

The cells were rinsed through a 100 µm cell-strainer (BD Falcon) and thereafter centrifuged at 400 x g for 5 min. Then the cells were re-suspended in medium and centrifuged at 400 x g for 5 min. After this, the cells were re-suspended in medium and placed in cell culture bottles (T-75)

coated with 0.2% gelatine (Sigma) The cells were cultured until confluence and thereafter harvested and stored frozen in liquid nitrogen.

The thawed cells were used for treatment in the second passage. The ECC cells were cultured in 6-well plates for RNA determination and ELISA analysis. When the cells were confluent, they were treated with lignocaine at a final concentration of 0.1 mg/ml or 1.0 mg/ml. The treatments were performed in triplicates and the cells were cultured for 24h and 48h. For ELISA, the culture medium was stored in -70°C until assay. The cells were harvested using lysis buffer with β-mercapto ethanol (Qiagen) and stored in -70°C until assay.

#### 4.5.2.2 *Cells from the peritoneal fluid*

Peritoneal fluid (PF) was collected aseptically before initiation of surgical procedures. Teflon catheters (tetrafluoro ethylene, diameter 1.7 mm; Optinova, Godby, Finland) and bottles of Teflon (Nalgene, Brochester, NY, USA) were used to prevent macrophages to adhere to the surfaces. Heparin (100 IE/ml) was supplemented to the PF to an estimated concentration of 10 IE/ml to avoid coagulation. The PF samples were transported to the laboratory at Uppsala University Hospital and were isolated within 3 hours.

The PFs were centrifuged at 2000 x g for 5 minutes and the cells were then re-suspended in RPMI 1640 medium (Life technologies). Mononuclear cells were thereafter isolated by using gradient centrifugation at 2000 x g for 15 min (Ficoll-Paque Plus, GE Healthcare Biosciences AB). After that, the cells were suspended in RPMI 1640, centrifuged at 2000 x g for 5 minutes and subsequently re-suspended in medium (RPMI 1640 supplemented with 1% L-glutamate (Life technologies), 1% Penicillin Streptomycin (Life technologies) and 1% foetal calf serum (Life technologies). The cells were dispensed and allowed to adhere in two wells in a 6 well plate for 45 minutes in an incubator at 5% $CO_2$, 37°C. Non-adherent cells were removed by washing three times with RPMI and the adherent cells were then cultured for 24 h in medium (RPMI 1640 supplemented with 1% L-glutamate (Life technologies), 1% Penicillin Streptomycin (Life technologies) and 1% foetal calf serum (Life technologies) with and without lignocaine at a final concentration of 0,1mg/ml. After 24 h, the supernatants were collected and kept at -70°C until ELISA assay. Lysis buffer (Quiagen) was added to adherent cells, which were thereafter stored at -70°C.

### 4.5.3 **Proliferation and viability assay on cells from ECC**

For determination of viability and proliferation, the ECC cells were cultured in 96-well plates to confluence and thereafter treated with lignocaine or control medium. Lignocaine treatment was performed at five different concentrations: 0.05 mg/ml, 0.1 mg/ml, 0.5 mg/ml, 1 mg/ml, and 1.5 mg/ml. Two different exposure times, 24h and 48 hours were used. Each treatment was performed in ten wells.
Cell proliferation is a measurement of the number of cells that are dividing in a culture and were measured using a BrDU (5-bromo-2-deoxyuridine) ELISA. Metabolic activity can be assayed as an indication of cell viability and the WST-1 assay (the ability of viable cells to cleave tetrazolium salts) was used for this measurement. The viability and proliferation

assays were carried out after lignocaine exposure according to the instructions from the manufacturer (Roche Diagnostics Scandinavia AB, Bromma, Sweden).

### 4.5.4 RNA isolation, cDNA synthesis and RT-PCR

RNA was isolated from lysed cells (cells from ECC and PF macrophages) using RNeasy Mini kit from Qiagen according to the instructions from the manufacturer. RNA concentration and purity was determined using NanoDrop. The quality of total RNA was determined using Agilent 2100 Bioanalyzer (Agilent Technologies, Sweden AB, Kista, Sweden). The RIN (RNA-integrity number) values differed between 2.7 and 9.4 for the RNA from PF macrophages and were 9.9-10 for RNA from ECC cells. All RNA sample were used for QPCR.
Synthesis of cDNA was performed by using SuperScript™ III Reverse Transcriptase First-Strand cDNA Synthesis (RT) (Invitrogen).
Quantitative PCR (QPCR) of cDNA was performed using TaqMan Real-time PCR (Applied Biosystems) for the cytokines IL-6, IL-8 and MCP-1, using 18S as housekeeping gene. The primers used were for IL-6 Hs00985639, for IL-8 Hs00174103 and for MCP-1/CCL2 Hs 00234140, all from TaqMan Gene Expression, Applied Biosystems.

#### 4.5.4.1 *The Real time-PCR principle*

TaqMan reagents use a fluorogenic probe to detect a specific PCR product as it accumulates during the PCR. With each PCR cycle, more reporter dye molecules are cleaved from their respective probes during primer extension, resulting in an increase of fluorescence intensity. This fluorescence intensity is proportional to the quantity of produced amplicon. The higher the starting copy number of the nucleic acid target the earlier a significant increase in fluorescence is observed. The cycle threshold (CT) is the number of cycles needed to reach the particular threshold fluorescence signal level (182). An internal reference gene (whose expression should be constant) can be used to compare samples and thereby compensate for differences in the amount of biological material in the tested samples.

We used 18S as internal reference for comparative quantification and calculated the relative gene expression using the ΔΔCT method (182).

For ECC cells also the standard curve method was used (to calculate the exact amount of RNA) but only the relative expressions are presented due to large spread in the RNA concentrations between samples.
The standard curve method could not be used for the macrophages due to lower amount of RNA and only relative expression in relation to 18S was calculated and presented.

### 4.5.5 ELISA on cell culture medium

Concentration of the cytokines MCP-1, IL-6 and IL-8 in culture media from ECC cells and PF macrophages were analysed using an ELISA according to the instructions from the manufacturer (Human MCP-1 ELISA, Human IL-6 ELISA and Human IL-8/CXCL8

ELISA, Biosensis). The concentrations of MCP-1, IL-6 and IL-8 were related to the total RNA concentration in each sample.

#### 4.5.5.1 *The ELISA principle*

The used ELISAs were sandwich ELISAs. The plates were precoated with antibodies specific for the cytokine that was to be detected. Samples and standards is added and then a secondary antibody specific for the cytokine. After washing, an enzyme complex is added which binds to the secondary antibody and peroxidase substrate is then added to induce a coloured reaction product. The intensity of the colour is proportional to the concentration of the cytokine in the sample.

### 4.5.6 **Cell staining**

In order to classify the ECC cells as of epithelial or stromal origin a cytochemical staining was performed. The ECC cells from five women were cultured on cover glass. The cells were fixed in paraformaldehyde, 4%, for 15 minutes, washed with PBS x3, permeabilised in cold methanol for 5-10 minutes at -20ºC and again washed in PBS x3. Thereafter, the cells were incubated with mouse anti-human cytokeratine (Clone AE1/AE3 DAKO Corporation) or rabbit anti-human vimentin (ab16700, Abcam) at 4°C overnight. After that, a fluorescent secondary antibody (Fluorescein anti mouse IgG or DyLight 594 anti-rabbit, Vector laboratories Inc, CA, USA) was added and the cells were incubated for 30 min at room temperature. The staining was evaluated using a fluorescence microscope (Zeiss, Axio Observer.Z1).

## 4.6 **STATISTICS**

For statistical analysis, Statsoft Statistica 10, Statsoft Statistica 12 and Microsoft Excel 2007 were used. Mostly non-parametric tests have been used due to ordinal scale variables and the relative small sample sizes.

In *study I*, the results were dichotomised and the success rate between the treatment and the placebo groups at different time points was compared using Fisher's exact test. The 95% confidence interval was requested by reviewers and considered an exploratory analysis since the study population was too small to assume normal distribution. In this thesis, also the change on the VAS scale from baseline to different follow up time points are compared between groups with Mann Whitney U test as an exploratory statistics.

In *study II*, only descriptive statistics were used.

In *study III*, the changes in the different dimensions on the EHP-30, from baseline to the follow up time points were compared between the treatment and the placebo groups with Mann Whitney U test (MWU test). Furthermore, the changes in the EHP-30 scores in the lignocaine and the placebo groups respectively were compared with the proposed MID and the proportion of patients improving more than the MID are reported. The purpose was to evaluate whether the improvement in quality of life could be perceived by the patients or not. The change in social

support scale after six months for those who improved /not improved >50% on VAS scale after four months was compared with MWU-test.

In *study IV*, the responsiveness to change for the EHP-30 questionnaire was evaluated with effect sizes and significance of change (paired t-test) in the improved and the stable groups. All the patients that were better or pain free (improved group) were compared with all the patients that felt the same or worse (stable group) independent of treatment group (lignocaine or placebo). The changes in EHP-30 scores between the improved and the stable groups were compared with independent student t-test.
In addition to the above parametric tests, complementary statistical analyses with non-parametric tests have been performed in this thesis. Mann Whitney U test was used for the differences in change between the improved and the stable groups and Wilcoxon signed ranks test for matched pairs for significance of change within groups.
The effect size was calculated by dividing mean difference with pooled SD i.e. Cohen *d* (156). Negative effect sizes correlates to improved quality of life in our material since a lower score indicate better Quality of Life.
The MIDs for scores on EHP-30 correlate to the mean change for patients who felt "somewhat better" (170). A negative value on the mean change means the EHP-30 score is lower at the follow up and thus that the patients are improved considering their quality of life.

In *study V*, Wilcoxon signed ranks test for matched pairs was used for data on cell viability and proliferation. The different concentrations of lignocaine were compared with the control (0 mg/ml) as well as with the next higher concentration at 24 and 48h respectively.
The relative gene expression and protein synthesis of MCP-1, IL-6 and IL-8 for cells treated with lignocaine were compared to untreated cells using Wilcoxon matched paired test.
Relative expression was calculated using the ΔΔCT method (Delta-Delta-Cycle Threshold-method) (182) and the control were set to 1 since $2^0=1$. The means of triplicate from each sample were used.
Differences between cell types were compared using Mann Whitney U test.

# 5 RESULTS

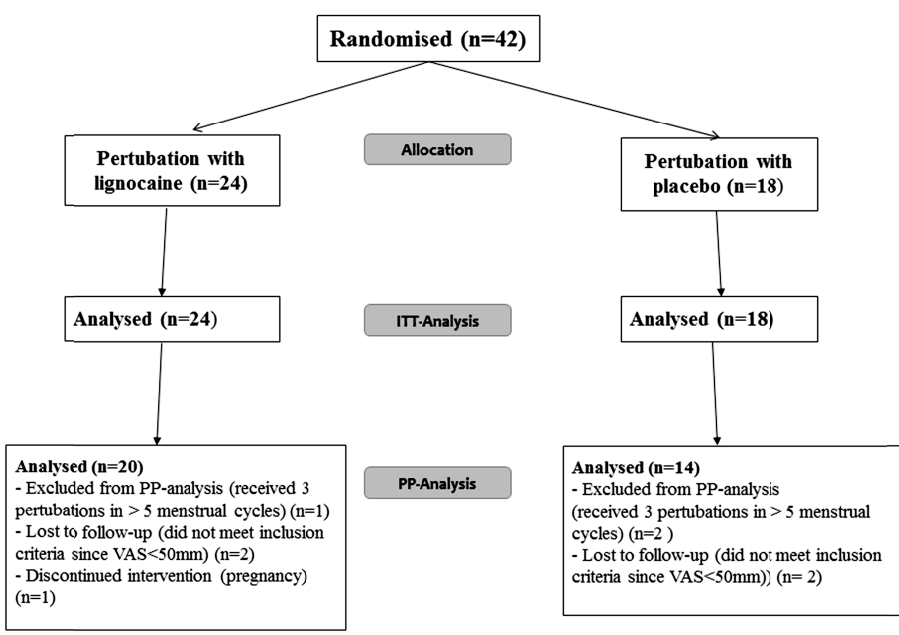
## 5.1 STUDY I;

The change in pain intensity on the VAS scale was evaluated after pertubation with lignocaine or placebo. In total, 124 pertubations, 70 with lignocaine and 54 with placebo, were carried out from March 22, 2007 to June 3, 2009. The last follow-up questionnaire was received on March 1st, 2010. In total, 42 patients were randomised, 24 to pertubation with lignocaine and 18 to placebo. The trial ended when sufficient number of participants had been recruited, based on the power analysis and with compensation for drop-outs.

The medical history and demographic characteristics of the patients in both groups were comparable (3.2.3. Demographics). They had similar usage of concomitant medications such as analgesics, selective serotonin reuptake inhibitors (SSRI), and oral contraceptives (OC).

The results of the pertubations with lignocaine or placebo were analysed in the PP (n=34) and the ITT populations (n=42) respectively. For inclusion in the PP analysis the subjects had to have a VAS score of >50mm, have undergone three pertubations in a maximum of five consecutive menstrual cycles and at least the primary endpoint evaluation had to be completed. Eight patients were excluded from PP analysis. Four initially reported VAS of >50mm but later when the baseline questionnaire where analysed proved not to fulfil this inclusion criterion. One subject became pregnant immediately after the first treatment and three further patients did not undergo treatments according to Per Protocol criteria. Thus 20 patients in the treatment group and 14 patients in the placebo group remained for the PP analysis (figure 4).

**Figure 4; Flow chart study I**



It was not possible to give the three treatments consistently during three consecutive cycles for different reasons such as bacterial vaginosis and because study patients were occasionally on vacation. However, the majority of the patients obtained their treatments over a period of three months (n=29) or within five months (n=38).

### 5.1.1 Primary end-point

The time point for primary efficacy evaluation was after the third and last pertubation that corresponded to the fourth menstruation approximately three months after the initial treatment. For the ITT analysis, 10 of 24 patients (41.7%) in the treatment group versus 3 of 18 patients (16.7%) in the control group fulfilled the criteria for success as having a decrease of ≥50% on the VAS scale (p=0.10, 95 % CI -7.26 to 36.2%). In the PP analysis, 9 of 20 (45.0 %) compared to 1 of 14 (7.1%) had a decrease of ≥50% on the VAS scale (p=0.024, 95 % CI -2.61 to 44.8%).

With other definitions of success, i.e. a decrease on the VAS scale of ≥40mm or VAS below 20mm at a specific time point, similar results were obtained (table 3).

**Table 3; Success rate on VAS scale at primary end-point**

| **Definition of success** | **population** | **Placebo Success rate** | **Lignocaine Success rate** | **p-value Fisher´s exact** |
|---|---|---|---|---|
| Decrease in VAS ≥ 50% | ITT | 3/18 | 10/24 | 0.10 |
| | PP | 1/14 | 9/20 | **0.024** |
| Decrease in VAS ≥40mm | ITT | 2/18 | 8/24 | 0.15 |
| | PP | 1/14 | 8/20 | 0.050 |
| VAS ≤ 20mm | ITT | 2/18 | 7/24 | 0.26 |
| | PP | 0/14 | 6/20 | **0.031** |

In the PP population, nine of the patients in the lignocaine group (n=20) and one in the placebo group (n=14) improved by ≥50% on the VAS scale from baseline to the first menstruation after the third treatment. In the lignocaine group, three of these nine patients were improved by ≥50% on the VAS scale after the first treatment and five after the second treatment. Three months after the third treatment, four of these nine patients in the lignocaine group still fulfilled the criterion of an improvement of ≥50% on the VAS scale from base line. This improvement persisted for six months after the last treatment for two patients and for nine months for four patients. The only patient in the placebo group, who improved by ≥50% after the third treatment, had not improved after the first or second. She was still improved three months after the third treatment, but thereafter her pain level was back at baseline (table 4). Also in this extended analysis of the improved patients in the PP population, the results were equal and independent of the definition of success.

Six patients in the lignocaine group achieved the secondary end point <20 mm on the VAS-scale compared to none in the placebo group (p= 0.031). Five of the nine subjects (56%) who responded to the treatment in the lignocaine group had no pain symptoms (VAS ≤4 mm). No

patient in the placebo group reached VAS of ≤10 mm at the time point for primary efficacy evaluation.

**Table 4; Successful treatments in the PP population after three pertubations**

| **Time point** | | After the first treatment | After the second treatment | **Success, first menstrual period after the third treatment** | 3rd menstrual period after the third treatment | 6th menstrual period after the third treatment | 9th menstrual period after the third treatment |
|---|---|---|---|---|---|---|---|
| **Definition of success** | | | | | | | |
| Improved **≥40 mm** on VAS scale from baseline | Lignocaine n=8 | 3 | 6 | **8** | 5 | 3 | 4 |
| | Placebo n=1 | 0 | 0 | **1** | 1 | 0 | 0 |
| Improved **≥50 %** on VAS scale from baseline | Lignocaine n=9 | 3 | 5 | **9** | 4 | 2 | 4 |
| | Placebo n=1 | 0 | 0 | **1** | 1 | 0 | 0 |
| VAS **≤20 mm** at specific time point | Lignocaine n=6 | 2 | 2 | **6** | 3 | 2 | 2 |
| | Placebo n=0 | 0 | 0 | **0** | 0 | 0 | 0 |

### 5.1.2 Secondary end-points

When analysing the sum of scores from the categorical scales during menstruation between baseline and the primary time-point after the third treatment, there were no significant differences between the groups.

The participating patients were asked to estimate any changes in their overall pain level, and 13 of the 24 patients in the lignocaine group and 11 of 18 in the placebo group reported reduced dysmenorrhea after the third treatment. Four patients in the lignocaine group experienced no dysmenorrhea at all. The change in the use of analgesics from baseline to primary time-point was comparable between the groups, and no significant changes were found. Only half of the study population reported a need to be on sick leave. The change in work capacity was similar between the groups.

Blood pressure and heart frequency recorded before pertubation were normal and did not change in either the lignocaine or the placebo group following treatment.

The secondary time points for primary efficacy evaluation were after 6, 9, and 12 months respectively. No significant differences in success rate were found on the VAS scale between the treatment group and the placebo group either in the ITT or in the PP population (table 5). Neither were there any differences between groups when analysing the sum of scores from the categorical scales at the secondary end-points.

**Table 5; Success rate on VAS scale at secondary end-points**

| | | After six months | | After nine months | | After twelve months | |
|---|---|---|---|---|---|---|---|
| **Definition of success** | **Population** | **Lignocaine** | **Placebo** | **Lignocaine** | **Placebo** | **Lignocaine** | **Placebo** |
| Decrease in VAS ≥ 50% | ITT | 4/17 | 4/14 | 2/14 | 3/12 | 4/14 | 2/9 |
| | PP | 4/16 | 3/11 | 2/14 | 1/9 | 4/13 | 1/7 |
| Decrease in VAS ≥40mm | ITT | 5/17 | 5/14 | 3/14 | 3/12 | 4/14 | 0/9 |
| | PP | 5/16 | 3/11 | 3/14 | 1/9 | 4/13 | 0/7 |

Additional statistics have been made in this thesis using the VAS scale as a continuous variable. The improvement on the VAS scale was significantly larger in the lignocaine group nine months after the third pertubation compared to the placebo group and the difference was significant with Mann Whitney U-test (p=0.04). There were no significant differences at the other time points and the spread between patients were large (table 6).

**Table 6; Change on VAS scale at all time points**

| **Time-point** | **Population** | **Placebo** Median(IQR) Min-max | **Lignocaine** Median(IQR) Min-max | **p-value MWU-test** |
|---|---|---|---|---|
| Four months | ITT | 23.5 (11.25-31.75) (-27)-71 | 30 (8.5-55) (-8)-88 | 0.52 |
| | PP | 23.5 (11.25-31.75) (-27)-71 | 34.5 (8,75-58,75) (-8)-88 | 0.29 |
| Six months | ITT | 14 (-4-40) -29-56 | 11.5 (2-27.5) (-26)-91 | 0.96 |
| | PP | 11.5 (-3.5-39) (-29)-56 | 18 (3-44) (-26)-91 | 0.84 |
| Nine months | ITT | 8 (0-29) (-28)-51 | 10 (0-38) (-19)-64 | 0.62 |
| | PP | 6 (0-17.25) (-7)-51 | 21 (3-40.5) (-13)-64 | 0.29 |
| Twelve months | ITT | 1 (-4-7) (-31)-35 | 19 (4-38.5) (-7)-72 | **0.04** |
| | PP | 4 (-4-9.5) (-31)-35 | 19.5 (6-42.25) (-3)-72 | **0.04** |

## 5.2 **STUDY II:**

A total of 97 serum samples were collected from 25 patients of whom 16 had been treated with lignocaine hydrochloride 10 mg and nine with placebo (Ringer Acetate). Due to problems with the peripheral venous catheter, samples could not be taken from one patient in the lignocaine group after 0 and 30 minute and also a 30 minutes sample is missing in the placebo group.

All patients were healthy and without cardiovascular or hepatic disease that might affect the pharmacokinetics of lignocaine. Baseline data and medication for patients included in the serum screening can be seen at 3.2.3. Demographics.

Overall low levels of lignocaine were detected in the serum samples following pertubation with lignocaine hydrochloride 10 mg. The highest observed concentration was 531 nM, which corresponds to 0.124 µg/ml and was seen after 30 minutes (table 7, figure 5).

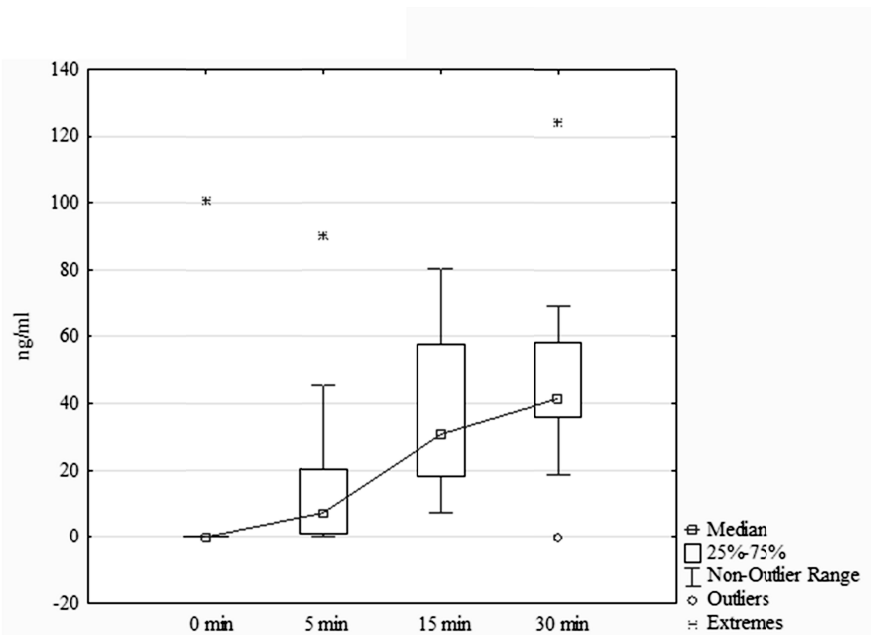
Of 16 patients, 14 had the highest level in the last sample i.e. after 30 minutes. One had the highest level after 5 minutes and one after 15 minutes. Time to maximum concentration ($T_{max}$) and maximum concentration ($C_{max}$) could not be calculated since the highest values were observed in the 30 min samples (table 7, figure 5).

Lignocaine was not found in any of the serum samples after pertubation with placebo (nine patients).

**Table 7; Serum concentration of lignocaine after pertubation**

| Time point | Concentration of Lignocaine in ng/ml | | | |
|---|---|---|---|---|
| | Mean (SD) | IQR | Median | Min-Max |
| 0 min | 0 (0) | 0-0 | | 0-0 |
| 5 min | 16.1 (23.4) | 1.1-20.5 | 7.3 | 0-90.2 |
| 15 min | 38.0 (25.1) | 18.3-57.9 | 30.8 | 7.3-80.4 |
| 30 min | 49.7 (24.8) | 36.6-59.3 | 43.3 | 18.7-124 |

**Figure 5; Serum concentration of lignocaine**



In total, 166 gynaecological examinations were carried out during the study, 42 of which were screening visits and 124 were treatment visits. There were no adverse events related to the treatment with lignocaine. Blood pressure and heart frequency recorded before pertubation were normal and did not change in either the lignocaine or the placebo group following treatment. A mild discomfort was experienced during the pertubation process at 11 of 124 treatments.

## 5.3 **STUDY III**

The change in quality of life was evaluated after pertubation with lignocaine (n=24) or placebo (n=18).

The groups were well matched in all demographic parameters and the pain intensity on the VAS scale was comparable. Two patients in each group initially reported a pain level on the VAS scale of >50mm but later when the baseline questionnaire where analysed proved not to fulfil this inclusion criterion. The groups had similar usage of concomitant medications such as selective serotonin reuptake inhibitors (SSRI), and OC (table 2). All patients used analgesics when needed.

The ITT population consisted of 42 patients. The results were similar in the PP and ITT population and therefore only the results from the ITT population are presented.

Only the complete scores are presented for the different dimensions giving different number of patients in various dimensions. One patient did not fill in the EHP-30 at baseline and was excluded from further analyses. Flow chart of participants and follow up can be seen in figure 6.

All scores on the different dimensions on the EHP-30 are ranging from 0 (best health status) to 100 (worst health status). A decrease on a score scale (i.e. negative change) at follow up implies that the patient has improved considering this dimension on quality of life.

The baseline quality of life was similar between the lignocaine and the placebo groups and there were no significant differences with MWU test (table 8).

**Figure 6; Flow chart study III**

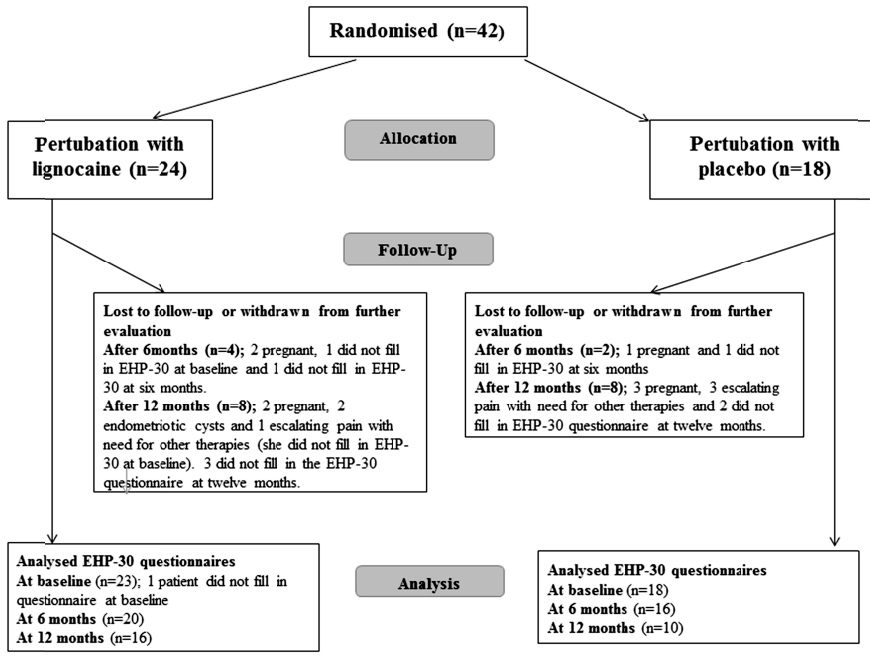



**Table 8; Baseline data from the EHP-30 questionnaire**

| EHP-30 dimension | Lignocaine | | | Placebo | | |
|---|---|---|---|---|---|---|
| | Mean(SD) | Min-Max | Number | Mean(SD) | Min-Max | Number |
| **Pain** | 51.7 (20.0) | 13.6-95.5 | n=23 | 50.8 (19.9) | 18.2-81.8 | n=17 |
| **Control and Powerlessness** | 59.6 (23.5) | 8.3-100 | n=23 | 67.1 (17.9) | 25-83.3 | n=18 |
| **Emotional well-being** | 54.2 (15.8) | 25-83.3 | n=20 | 53.7 (18.1) | 8.3-91.7 | n=18 |
| **Social support** | 52.3 (22.6) | 0-93.8 | n =22 | 47.9 (20.8) | 18.8-93.8 | n=18 |
| **Self Image** | 34.1 (17.6) | 0-58.3 | n=22 | 25.5 (18.4) | 0-58.3 | n=18 |
| **Sexual intercourse** | 41.8 (27.3) | 0-100 | n=21 | 41.1 (24.1) | 0-80 | n=17 |

After six months from the first treatment (i.e. two-three months after the last treatment) there was a significant difference between the lignocaine and the placebo groups on the EHP-30 questionnaire for the dimension social support. There were no differences between the groups for the other dimensions after six months (table 9). After twelve months there were no differences between the lignocaine and the placebo groups concerning the change in EHP-30 in any dimension, hence the significant effect seen at six months regarding social support disappeared (table 9).

**Table 9; Change in the EHP-30 questionnaire**

| **EHP-30 dimension** | **Change after six months** | | | **Change after twelve months** | | |
|---|---|---|---|---|---|---|
| | **Lignocaine** | **Placebo** | MWU test | **Lignocaine** | **Placebo** | MWU test |
| | Median<br>IQR | Median<br>IQR | *p*-value | Median<br>IQR | Median<br>IQR | *p*-value |
| **Pain** | -13.6<br>(-27.3)-2.3<br>n=20 | -11.4<br>(-22.7)-(-2.3)<br>n=15 | 0.99 | -8.0<br>(-29.5)-2.3<br>n=14 | -11.4<br>(-20.5)-(-4.5)<br>n=9 | 0.69 |
| **Control and Powerlessness** | -8.3<br>(-33.3)-2.1<br>n=20 | -6.3<br>(-35.4)-2.1<br>n=16 | 0.84 | -12.5<br>(-37.5)-(-8.3)<br>n=13 | -20.8<br>(-41.7)-0<br>n=10 | 0.74 |
| **Emotional well-being** | -4.2<br>(-37.5)-4.17<br>n=18 | -12.5<br>(-20.8)-6.25<br>n=16 | 0.99 | -20.8<br>(-37.5)-0<br>n=12 | -12.5<br>(-25.0)-4.17<br>n=10 | 0.63 |
| **Social support** | -18.8<br>(-31.25)-0<br>n=19 | -6.3<br>(-12.5)-6.25<br>n=16 | **0.034** | -12.5<br>(-37.5)-0<br>n=15 | -6.3<br>(-31.25)-12.5<br>n=10 | 0.50 |
| **Self image** | -8.3<br>(-16.7)-0<br>n=19 | 0.0<br>(-16.67)-8.33<br>n=16 | 0.24 | -8.3<br>(-16.7)-0<br>n=15 | 0.0<br>(-16.7)-0<br>n=10 | 0.57 |
| **Sexual intercourse** | -10.0<br>(-25.0)-10.0<br>n=15 | 5.0<br>(-10)-5<br>n=14 | 0.24 | -7.5<br>(-15.0)-5<br>n=12 | -7.5<br>(-20.0)-7.50<br>n=8 | 0.97 |

When comparing the mean changes on the EHP-30 questionnaire after six and 12 months with the minimal important differences (MID), the levels were surpassed for more dimensions in the lignocaine group compared to the placebo group (table 10). The fact that the mean change exceeds the MID does not mean that all patients are improved and the proportion of patients improving above the MID can be seen in table 10. A significant difference between lignocaine and placebo groups were found for the social support scale when comparing the proportion of patients improving more than the MID (Fisher exact test, p=0.036)

**Table 10; Mean change on the EHP-30 and proportion improved above the MID**

| | | **After six months** | | **After twelve months** | |
|---|---|---|---|---|---|
| | | **Lignocaine** | **Placebo** | **Lignocaine** | **Placebo** |
| **EHP-30 dimensions** | **MID Mean(SD)** | Mean(SD)<br>Proportion improved > MID | Mean(SD)<br>Proportion improved > MID | Mean(SD)<br>Proportion improved > MID | Mean(SD)<br>Proportion improved > MID |
| **Pain** | -19.9 (18.8)<br>n=17 | -14.4(22.5)<br>6/20 | -12.0(15.0)<br>4/15 | -14.6(22.0)<br>4/14 | -13.4(14.8)<br>3/9 |
| **Control and powerlessness** | -25.7 (25.9)<br>n=17 | -18.5(27.2)<br>8/20 | -16.9 (25.6)<br>5/16 | -26.0(24.5)<br>6/13 | -20.8(21.8)<br>3/10 |
| **Emotional well-being** | -13.9 (21.4)<br>n=15 | -10.0(25.8)<br>5/18 | -8.1(22.7)<br>8/16 | -19.8(24.3)<br>7/12 | -15.4(25.1)<br>5/10 |
| **Social support** | -12.9 (18.0)<br>n=17 | -18.8 (20.3)<br>11/19 | -3.1(15.0)<br>3/16 | -17.9(22.4)<br>7/15 | -10.63(31.0)<br>4/10 |
| **Self image** | -6.9 (15.6)<br>n=17 | -7.9 (17.0)<br>10/19 | -0.52(13.8)<br>6/16 | -8.9 (12.4)<br>9/15 | -5.8(18.0)<br>4/10 |
| **Sexual intercourse** | -4.5 (27.8)<br>n= 14 | -8.2 (25.4)<br>8/15 | -0.0(21.1)<br>5/14 | -5.6 (22.6)<br>9/12 | -5.0(17.7)<br>5/8 |

To evaluate the authenticity of the results seen on social support scale some additional calculations were done. The relation between the change in pain level immediately after three pertubations and the effect on social support scale two months later was investigated with data from study I. Those who improved on the VAS scale >50% after four months (lignocaine n=10, placebo n=3) had significantly better score on social support after six months ($p$=0.005, MWU-test) compared to those who did not improve on the VAS scale (lignocaine n=9, placebo n=13). The difference concerning the change on the social support scale between these groups was however not significant ($p$=0.32, MWU-test). The baseline VAS did not differ significantly between those groups ($p$=0.43, MWU-test) but there was a tendency to better social support scale ($p$=0.09, MWU-test) at the baseline in the improved group.

### 5.3.1 **Study I-III withdrawals**

Five patients became pregnant during the study and were withdrawn from further evaluation. Two patients in the lignocaine group achieved spontaneous pregnancy after the first and third pertubation respectively. Three patients in the placebo group became pregnant after IVF. The pregnancies were normal. One malformation was registered in the placebo group after IVF treatment and the other children were healthy.

Two patients in the lignocaine group were withdrawn because of endometriomas >25 mm diagnosed one and four months after third treatment, respectively. They were surgically treated and withdrawn from further evaluation. Another patient in the lignocaine group discontinued five days after the third treatment because of such painful endometriosis that continuous OC had to be initiated. In the placebo group, three patients were withdrawn during the study due to escalating pain and need for other therapies such as high doses of gestagens or GnRH agonists.

## 5.4 **STUDY IV**

In total, 103 EHP-30 questionnaires were collected at different time-points, 41 at baseline, 36 after six months and 26 after 12 months. The demographic of the whole study population was as follows: the mean age was 33.2 (±5.1) years (minimum 22 and maximum 43 years) and the mean duration of endometriosis was 4.95 (±4.2) years (minimum 0 and maximum 16 years). Of the included patients 65% were nulliparous, 19% had delivered once and 14 % were multiparous with at least two children

The questionnaires collected after six months were used for the responsiveness analyses whereas the data completeness analysis also includes the questionnaires collected after twelve months.

Data completeness for the core scales was good. For the modular score sexual intercourse, data were complete in all 103 questionnaires but 11/103 (11%) could not be analysed since patients reported they did not have sexual intercourse for other reasons (table 11). The floor and ceiling effects were low. At the baseline, the analysed sample of 41 patients had the lowest score (i.e. best quality of life) on the dimension self image and sexual intercourse and highest score (i.e. worst quality of life) on the dimensions control and powerlessness and emotional well-being (table 11).

**Table 11: Baseline data EHP-30 and data completeness after six and twelve months**

| **EHP-30** | **Baseline** (n=41) | | | **Complete questionnaires** | |
|---|---|---|---|---|---|
| | Mean (SD)<br>Min-max | Floor | Ceiling | rate | % |
| **Pain**<br>Question 1-11 | 51.3 (19.7)<br>13.6-95.5<br>n=40 | 0/41 | 0/41 | 100/103 | 97 |
| **Control and powerlessness**<br>Question 12-17 | 62.9 (21.3)<br>8.3-100<br>n=41 | 0/41 | 1/41 | 100/103 | 97 |
| **Emotional well-being**<br>Question18-23 | 53.9 (16.7)<br>8.3-91.7<br>n=38 | 1/41 | 0/41 | 98/103 | 95 |
| **Social support**<br>Question24-27 | 50.3 (21.6)<br>0-93.7<br>n=40 | 1/41 | 0/41 | 102/103 | 99 |
| **Self-image**<br>Question28-30 | 30.2 (18.3)<br>0-58.3<br>n=40 | 2/41 | 0/41 | 102/103 | 99 |
| **Sexual intercourse**<br>QuestionC1-C5 | 41.5 (25.6)<br>0-100<br>n=38 | 3/41 | 1/41 | 92/103 | 89 |

On the pain-questionnaires, the participating patients were asked to estimate any changes in their overall pain level and after six months, 34 patients estimated their pain according to this question. One patient was excluded from analysis since she could not be classified as pain free, better, same or worse. The *improved* group consisted of the patients that were classified as better or pain free (n=19) and the non-improved or *stable* group of patients that were classified as same or worse (n=14).

The mean change on EHP-30 scores correlates with the patients' own estimation of change in pain intensity, indicating that the pain intensity has an effect on all dimensions of quality of life (table 12). The improvement or deterioration in the quality of life was related to the improvement or deterioration in the pain intensity.
The minimal important difference on the different EHP-30 scores in our material corresponds to the mean change for patients evaluating their pain to be better (n=17) and excluding the two that were pain-free (table 12).

**Table 12, Change in EHP-30 in relation to change in pain intensity after six months**

| EHP-30 | Worse n=8 | Same n=6 | Better= Minimal important difference n=17 | Pain free n=2 |
|---|---|---|---|---|
| | Mean[a] (SD) | Mean[a] (SD) | Mean[a] (SD) | Mean[a] (SD) |
| **Pain** | 3.7(10.4) n=8 | -10.5 (10.4) n=5 | -19.9 (18.8) n=17 | -39.8 (37.0) n=2 |
| **Control and powerlessness** | 4.2(13.0) n=8 | -10.4 (16.6) n=6 | -25.7 (25.9) n=17 | -50.0 (23.6) n=2 |
| **Emotional well-being** | 9.5(14.6) n=7 | 4.2 (20.7) n=6 | -13.9 (21.4) n=15 | -50.0 (17.7) n=2 |
| **Social support** | 0 (14.4) n=7 | -8.3 (17.5) n=6 | -12.9 (18.0) n=17 | -50.0 (26.5) n=2 |
| **Self image** | 4.8 (15.1) n=7 | -1.4 (12.3) n=6 | -6.9 (15.6) n=17 | -25 (23.6) n=2 |
| **Sexual intercourse** | 13.0 (16.0) n=5 | -3.0 (15.6) n=5 | -4.5 (27.8) n= 14 | -27.5 (3.5) n=2 |

[a] Negative values indicate improved Quality of Life

**Table 13: Effect size and significance of change in relation to change in pain intensity**

| EHP-30 | Worse and same n=14 | | | Better and pain-free n=19 | | |
|---|---|---|---|---|---|---|
| | Effect size[a] | p-value paired t-test | p-value Wilcoxon | Effect size[a] | p-value paired t-test | p-value Wilcoxon |
| **Pain** | -0.09 | 0.62 | 0.53 | -1.22 | **0.0002** | **0.0008** |
| **Control and powerlessness** | -0.10 | 0.63 | 0.62 | -1.24 | **0.0002** | **0.001** |
| **Emotional well-being** | 0.35 | 0.16 | 0.20 | -1.04 | **0.006** | **0.008** |
| **Social support** | -0.17 | 0.40 | 0,42 | -0.84 | **0.003** | **0.006** |
| **Self image** | 0.11 | 0.62 | 0.44 | -0.51 | **0.04** | **0.03** |
| **Sexual intercourse** | 0.15 | 0.38 | 0.25 | -0.30 | 0.29 | 0.30 |

[a] Negative values on effect size indicate improved Quality of Life

For the patients in the improved group (better and pain free), the change in EHP-30 scores was significant for all dimensions but sex. The effect sizes for changes were large for all dimensions except "self image" (moderate change) and "sex" (small change, table 13). Significance of changes and effect sizes were also calculated for patients in the stable group (same and worse). There were no significant changes in any dimension on the EHP-30 questionnaire in the stable group and the effect sizes were small indicating that there was little change in health status (table 13).

The change in the different EHP-30 scores between the improved (better and pain free) and the stable group (same or worse) after six months were compared with independent student t-test and Mann Whitney U test. There were significant differences for the change in EHP-30 scores for pain, control and powerlessness and emotional well-being whereas there was a tendency for significance for social support and self image (table 14). The difference was not significant for the dimension sexual intercourse.

**Table 14, Change in EHP-30 for improved and not improved (stable) group**

| | **Patients´ estimated change in pain intensity** | | | |
|---|---|---|---|---|
| **EHP-30** | **Worse and same** n=14 Mean (SD) | **Better or pain-free** n=19 Mean (SD) | p-value independent t-test | p-value Mann Whitney U test |
| **Pain** | -1.7 (12.3) n=13 | -22.0 (20.7) n=19 | **0.004** | **0.040** |
| **Control and powerlessness** | -2.1 (15.9) n=14 | -28.3 (26.2) n=19 | **0.002** | **0.006** |
| **Emotional well-being** | 7.1 (17.1) n=13 | -18.1 (23.8) n=17 | **0.003** | **0.005** |
| **Social support** | -3.8 (15.8) n=13 | -16.8 (21.6) n=19 | 0.07 | 0.08 |
| **Self image** | 1.9 (13.7) n=13 | -8.8 (16.8) n=19 | 0.07 | 0.13 |
| **Sexual intercourse** | 5 (17.2) n=10 | -7.3 (27.0) n=16 | 0.21 | 0.29 |

The stable group was separated into two small subgroups and the effect sizes were calculated in the group that was "same" i.e. unchanged (n=6) and worse, (n=8) respectively.
There were no significant changes in any EHP-30 score in the same group (p=0.08-0.64) or in the worse group (p=0.13-1). The effect sizes were small in both subgroups except on two scales in the subgroup that felt the same, in which the effect size were moderate for pain (-0.79) and for control and powerlessness (-0.70). Thus, some of the scales displayed responsiveness in the small group that felt the same but for the other dimensions the effect

sizes were small, indicating small changes in quality of life for patients reporting themselves to feel the same during periods.

## 5.5 STUDY V

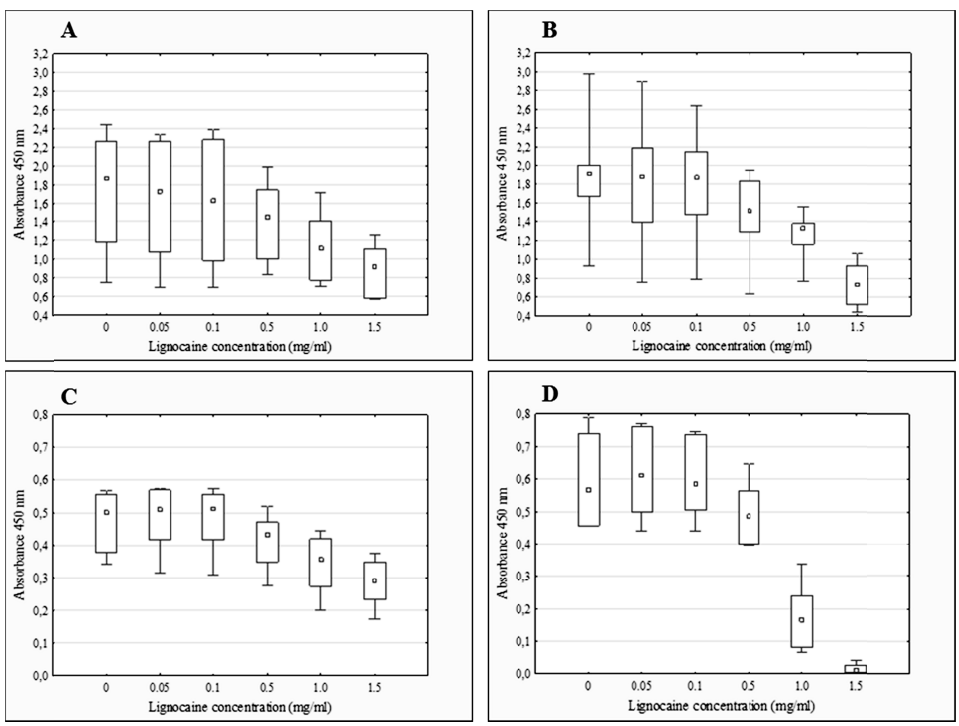
### 5.5.1 Proliferation and viability of ECC cells

Lignocaine caused decreased proliferation and viability in a dose and time dependent manner (figure 7). A significant decrease in proliferation and viability were seen at lignocaine concentrations ≥0.5 mg/ml after both 24h and 48h compared to untreated controls ($p<0.05$). A decrease in viability compared to 0 mg/ml was also seen at 0.05 mg/ml and 0.1 mg/ml after 24 h ($p<0.05$) but the proliferation at the same concentration and time-point, was not affected ($p=0.46$-$0.75$).

**Figure 7; Cell proliferation and viability in ECC cells after lignocaine treatment**

Cell proliferation and viability of ECC cells after incubation with lignocaine in different concentrations for 24h and 48 h showing Min-Max, Interquartile range and Median for absorbance at 450 nm.

**A**; Viability 24 h, **B**; Viability 48 h, **C**; Proliferation 24 h, **D;** Proliferation 48 h



### 5.5.2 **Effect of lignocaine on gene expression and protein levels**

#### 5.5.2.1 *MCP-1*

There were no significant differences in gene expression for MCP-1 in ECC cells after incubation with lignocaine 0.1 mg/ml for 24h (p=0.13) or 48h (p=0.87) compared to untreated cells at the same time point (table 15, figure 8). Neither were there any differences in protein levels of MCP-1 in culture media of ECC cells treated with lignocaine (24 or 48 h) compared to media from untreated ECC cells (p=0.13 and p=0.74, table 16 and figure 8).

The gene expression of MCP-1 was significantly lower (p=0.043) in macrophages from healthy women (n=5) treated with lignocaine compared to untreated macrophages from these healthy women. The macrophages from women with endometriosis (n=5) showed diverging results with attenuated expression of MCP-1 in two samples and the gene expression was not different from controls after 24h in this subgroup (p=0.69, table 15 and figure 8). The protein levels of MCP-1 in cell culture media were not different between cultures of macrophages supplemented with lignocaine compared to untreated macrophages (MØ healthy p=0.47, MØ Endo p=1, table 16 and figure 8).

**Figure 8; Relative gene expression and protein levels of MCP-1**

**A;** Box-plot showing Median, Interquartile range and Min-Max for the relative gene expression of MCP-1 in macrophages and ECC cells after incubation with lignocaine 0.1 mg/ml for 24 h. **B;** Box-plot showing relative protein levels of MCP-1 (lignocaine-treated divided with untreated) in macrophages and stromal cells after incubation with lignocaine 0.1 mg/ml for 24 h. *= significant with p-value <0.05

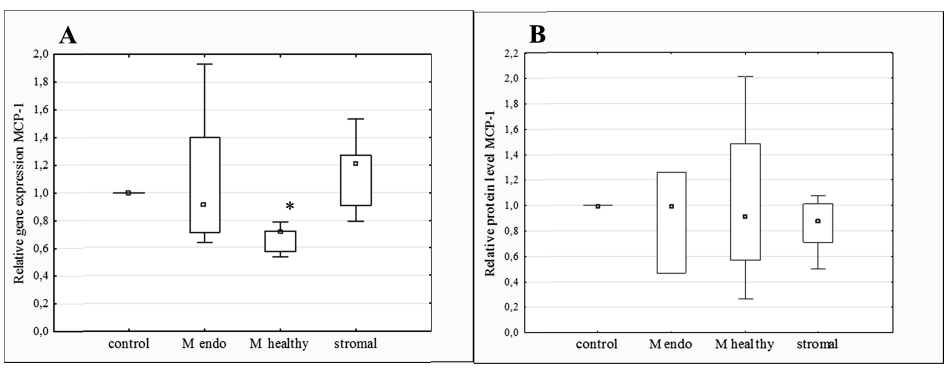


**Table 15: Relative gene expression**

Relative gene expression (ΔΔCT-value) of MCP-1, IL-6 and IL-8 in macrophages and ECC cells after incubation with lignocaine 0.1 mg/ml for 24 h and 48h. The relative gene expression for each cell type and cytokine was compared with its own control (untreated cells, relative expression =1)) with Wilcoxon signed rank test. MØ: macrophages, ECC cells: endometriotic cyst capsule cells

| RNA Relative gene expression | MØ endo. 24h N=5 | | MØ healthy 24h N=5 | | ECC cells 24h N=7 | | ECC cells 48h N=7 | |
|---|---|---|---|---|---|---|---|---|
| | **Rel. expr.** Median Min-max | p-value | **Rel. expr.** Median Min-max | p-value | **Rel. expr.** Median Min-max | p-value | **Rel. expr.** Median Min-max | p-value |
| MCP-1 | 0.91<br>0.64-1.93 | 0.69 | 0.71<br>0.53-0.78 | **0.043** | 1.21<br>0.80-1.53 | 0.13 | 1.02<br>0.69-1.44 | 0.87 |
| IL-6 | 0.65<br>0.16-1.09 | 0.08 | 0.72<br>0.35-0.87 | **0.043** | 0.94<br>0.77-1.36 | 0.87 | 1.00<br>0.73-1.17 | 0.74 |
| IL-8 | 0.86<br>0.62-1.84 | 0.50 | 0.50<br>0.36-0.88 | **0.043** | 0.87<br>0.55-1.02 | **0.03** | 0.94<br>0.78-1.16 | 0.31 |

**Table 16; Protein levels**

Protein levels correlated to RNA concentration (pg / µg RNA). Cells treated with Lignocaine 0.1 mg/ml for 24-48h were compared to controls ( i.e. untreated cells of the same cell type at the same time point) with Wilcoxon rank sum test for matched pairs . MØ: macrophages, ECC cells: endometriotic cyst capsule cells

| Protein levels (pg/µg RNA) | Cell types and treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | MØ endo. n=5 24h | | MØ healthy n=4 24h | | ECC cells n=7 24h | | 48h | |
| | Untreated | Lignocaine | Untreated | Lignocaine | Untreated | Lignocaine | Untreated | Lignocaine |
| MCP-1 | 9.31 | 4.36 | 5.13 | 3.84 | 2.80 | 1.83 | 2.21 | 2.39 |
| Median | 1.99-23.11 | 1.97-29.1 | 2.31-9.47 | 2.49-6.48 | 0.76-3.86 | 0.54-3.37 | 0.91-3.76 | 0.83-3.38 |
| Range | n=3 | n=3 | | | | | | |
| p-value | p=1 | | p=0.47 | | p=0.13 | | p=0.74 | |
| IL-6 | | | | | | | | |
| Median | 2.11 | 1.25 | 2.08 | 2.68 | 0.23 | 0.22 | 0.44 | 0.49 |
| Range | 1.02-4.51 | 0.79-4.60 | 1.54-5.05 | 1.19-4.02 | 0.10-0.78 | 0.15-0.52 | 0.12-0.98 | 0.14-1.78 |
| p-value | p=0.080 | | p=0.72 | | p=0.18 | | p=0.13 | |
| IL-8 | | | | | | | | |
| Median | 196.2 | 146.3 | 22.9 | 29.7 | 5.95 | 4.20 | 4.75 | 3.95 |
| range | 71.5-498.1 | 99.9-673.0 | 18.2-69.7 | 14.7-85.8 | 4.46-15.06 | 2.55-9.36 | 2.96-8.15 | 3.16-7.14 |
| p-value | p=0.69 | | p=0.27 | | **p=0.02** | | p=0.40 | |

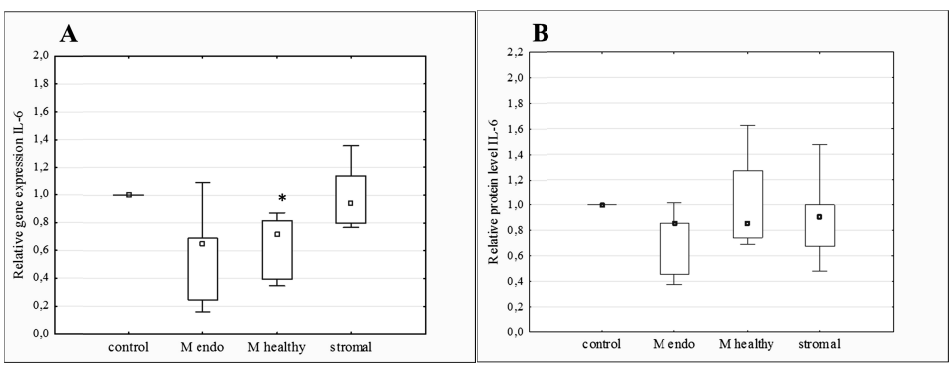
#### 5.5.2.2 *IL-6*

For ECC cells, the relative expression of IL-6 for cells treated with lignocaine 0.1 mg/ml after 24 or 48h were not different from controls at the same time point (p=0.87 and p=0.74, table 15 and figure 9). Neither did the concentration of IL-6 in cell culture media of ECC cells differ between cells cultured with or without lignocaine for 24 or 48 h (p=0.18 and p=0.13, table 16 and figure 9).

Macrophages from healthy women (n=5) showed a significantly lower gene expression of IL-6 after incubation with lignocaine compared to untreated controls (p=0.043). The gene expression of IL-6 in macrophages from patients with endometriosis (n=5) was not significantly different between treated and untreated cells (p=0.08) since attenuated expression of IL-6 was found in one of the samples (table 15, figure 9). The protein levels of IL-6 in cell culture media from macrophages after incubation with lignocaine 0.1 mg/ml showed no differences between treated and untreated cells even if there was a tendency for lower levels of IL-6 in macrophages from patients with endometriosis (p=0.08) (table 16, figure 9).

**Figure 9; Relative gene expression and protein levels of IL-6**

**A;** Box-plot showing Median, Interquartile range and Min-Max for the relative gene expression of IL-6 in macrophages and ECC cells after incubation with lignocaine 0.1 mg/ml for 24 h. **B;** Box-plot showing relative protein levels of IL-6 (lignocaine-treated divided with untreated) in macrophages and stromal cells after incubation with lignocaine 0.1 mg/ml for 24 h. *= significant with p-value <0.05



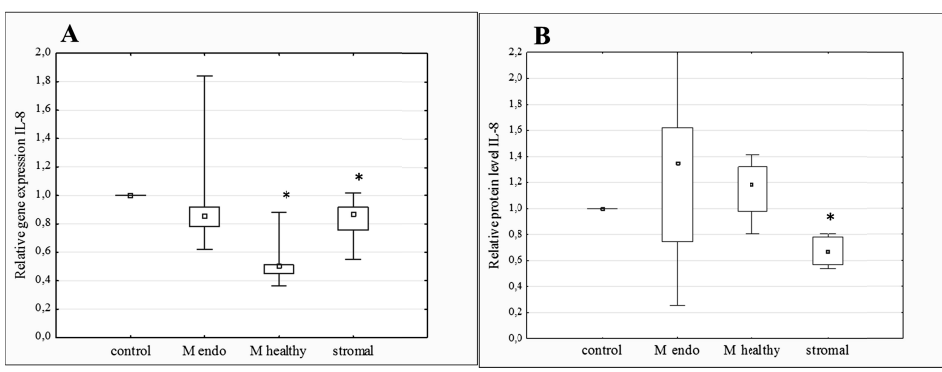
#### 5.5.2.3 *IL-8*

There was a significantly lower gene expression of IL-8 in ECC cells after 24h incubation with lignocaine 0.1 mg/ml (p=0.03) compared to the untreated cells (table 15, figure 10). Also the concentration of IL-8 was lower in cell culture media from ECC cells treated with lignocaine for 24h compared to untreated cell cultures (p=0.02, table 16 and figure 10). No differences were seen between treated and untreated ECC cells after 48 h incubation, neither on gene expression (p=0.31, table 15) or protein level of IL-8 (p=0.40, table 16).

A significant difference in gene expression of IL-8 was seen between lignocaine-treated and untreated macrophages from healthy women (n=5, p=0.04). The gene expression of IL-8 in macrophages from endometriosis patients was not different between the lignocaine-treated and untreated cells (p=0.50) since the gene expression of IL-8 was attenuated in one sample (table 16, figure 10). The protein levels of IL-8 in cell culture media after incubation of PF macrophages with lignocaine 0.1 mg/ml showed no significant differences (p=0.27 and p=0.69, table 16 and figure 10).

**Figure 10; Relative gene expression and protein levels of IL-8**

**A;** Box-plot showing Median, Interquartile range and Min-Max for the relative gene expression of IL-8 in macrophages and ECC cells after incubation with lignocaine 0.1 mg/ml for 24 h. **B;** Box-plot showing relative protein levels of IL-8 (lignocaine-treated divided with untreated) in macrophages and stromal cells after incubation with lignocaine 0.1 mg/ml for 24 h. *= significant with p-value <0.05



#### 5.5.2.4 *Differences between macrophages from healthy women and women with endometriosis*

Macrophages from patients with endometriosis produced significantly higher levels of IL-8 in cell culture media compared to macrophages from healthy women. This was seen both without and with the supplement of lignocaine (MWU-test, p=0.02, table 16). No difference were seen between macrophages from healthy women and macrophages from women with endometriosis in the protein levels of IL-6 or MCP-1 (p=0.73-0.86, table 16).
The relative gene expression and the relative protein secretion of the different cytokines (treated/untreated cells) were also compared between macrophages from healthy and endometriosis women. This corresponds to the cells reaction on lignocaine treatment. A tendency for different effect on gene expression was seen for IL-8 since macrophages from healthy women had lower relative gene expression compared to macrophages from patients with endometriosis (p=0.056, MWU test, table 15 and figure 10). There were no significant differences in relative gene expression between healthy and endometriosis macrophages for MCP-1 or IL-6 (p=0.22 and 0.55, figure 8 and 9). The relative protein secretion did not differ between macrophages from healthy or endometriosis women for any cytokine (p=0.56-0.90, figure 8-10).

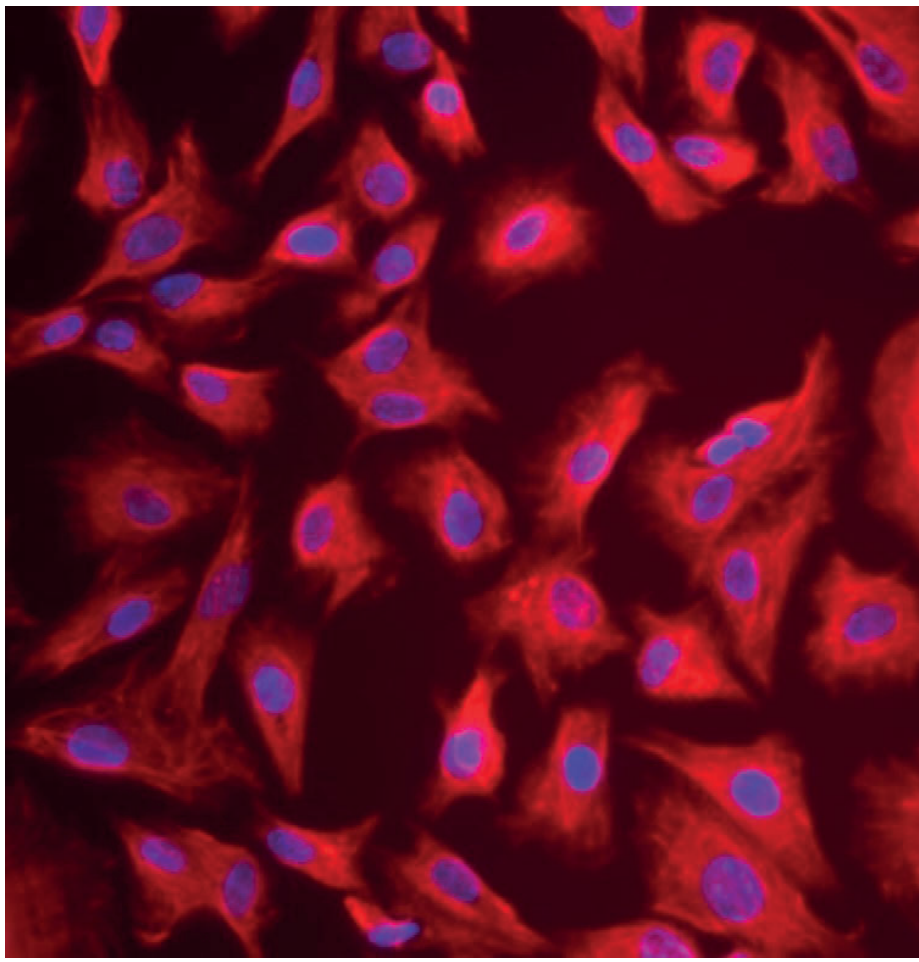
#### 5.5.2.5 *Differences between macrophages and stromal cells*

The macrophages from patients with endometriosis produced significantly more IL-6 and IL-8 in cell culture media compared to ECC cells both with and without the supplement of lignocaine (p=0.003, MWU-test, table 16). The protein levels of MCP-1 in culture media did not differ between macrophages and ECC cells (p=0.12-0.18).
When comparing relative gene expression and relative protein secretion (treated/untreated) between macrophages and ECC cells (MWU test) no differences were seen (gene expression p=0.10-0.88 and protein secretion p=0.22-0.83).

### 5.5.3 Cell staining on ECC cells

The presence of vimentin was seen in the five ECC cultures, while cytokeratin was not observed, indicating that the cells were of stromal origin (figure 11).

**Figure 11; Immunofluorescence staining of ECC cells** Immunofluorescence staining of ECC cells showing red perinuclear staining for vimentin indicating stromal origin.



# 6 DISCUSSION

## 6.1 GENERAL DISCUSSION

Endometriosis is a chronic disease treated surgically with resection of endometriotic tissue or pharmacologically (108). Hormonal therapeutic approaches currently used for amelioration of endometriosis-associated pelvic pain all inhibit ovulation, have side effects and do not improve subsequent spontaneous fertility (106).
There is a need for new treatment options for patients with endometriosis. An optimal new treatment should be efficacious on both pain symptoms and fertility. It should have few and acceptable side effects, not be contraceptive, not interfere with ovulation or the implantation capacity of the endometrium and not have teratogenic effects. A new treatment would preferably inhibit the growth and the development of endometriotic lesions and improve the chances of natural conception.

Pertubation with lignocaine represents an alternative non-hormonal treatment strategy. The first clinical trial of pertubations with lignocaine was carried out after an *in vitro* study on peritoneal macrophages (128). Patients with endometriosis, infertility and patent Fallopian tubes were offered pre-ovulatory pertubations in a double-blind cross-over study and 32 % of patients who did not become pregnant reported reduced dysmenorrhea (135). A separate study intended to investigate the effect on pain showed promising results since five of six patients treated with lignocaine 1mg/ml reported reduced dysmenorrhea (137). Lignocaine affects inflammatory cells but might also affect other cells involved in the pathophysiology of endometriosis such as glandular and stromal cells in eutopic and ectopic endometrium and sensory nerves.

The objective of the studies presented in this thesis was to further evaluate the effect of lignocaine pertubations on pain and quality of life, to evaluate the safety behind the method and to examine some effects of lignocaine *in vitro*.
The results indicate that pertubation with a low dosage of lignocaine can improve dysmenorrhea and quality of life in some patients with endometriosis. The procedure seems to be safe and without side effects related to lignocaine. The cell culture study suggests that lignocaine may affect the gene expression and secretion of some pro-inflammatory cytokines involved in endometriosis and this could be an explanation for the subtle clinical effect seen on dysmenorrhea and quality of life.
The optimal dosage and therapy intervals are not known but the pharmacokinetic study indicates that it would be safe to pertubate higher dosages of lignocaine.
In the following discussion both advantages and disadvantages in the included studies are addressed. Also a discussion around the results, implications and possible future studies will follow.

## 6.2 DISCUSSION PAPER I

The PP analysis demonstrated a significant pain reduction on the VAS scale after three pertubations with lignocaine compared to placebo but no significant effect was seen in the ITT population.

In the preceding study by Edelstam et al., the effect on dysmenorrhea was evaluated only with the patients' own estimation of change in pain intensity (137). In the presented RCT, the VAS scale was chosen for primary evaluation and the definition of success was set rather high since we wanted to exclude the placebo effect. This might be an explanation for the different rates of success between the small study on which the power calculation was based and the present thesis (83% and 42% respectively).

There are few studies defining criteria for relevant improvement on the VAS-scale. A randomised study evaluating the effect of laparoscopic uterine nerve ablation in women with chronic pelvic pain defined improvement as >50% on the VAS-scale from baseline (100). The expected placebo effect could probably be excluded if improvement was defined as >50% on the VAS scale, and this level was therefore used. In a study based on two small RCTs, the best separation between women rating themselves "minimally improved" and "improved" was found to be a decrease of 28 mm on the VAS-scale (163). Such a low definition of success did not allow a distinction of treatment effect from placebo effect in our study. The average strength of placebos upon pain on a visual analogue scale is 2 out of 10 units (176, 177). Individuals who respond to placebos may show even greater effects up to 5 out of 10 units (174).

When analysing the present results with other definitions of success, e.g. ≥ 40 mm decrease on the VAS scale or obtaining a VAS below 20mm, similar results were obtained as with improvement ≥ 50% on the VAS scale. Also these levels were assumed to exclude the placebo effect or the effect of reporting bias. Unpublished data indicate that the MID on the VAS scale in the present study population is a decrease around 37 mm or 50%. When using a decrease >37mm on the VAS scale as cut of, there are 9/24 in lignocaine and 2/18 in placebo group obtaining success in the ITT analysis (p= 0.080) and in the PP population the results are 9/20 and 1/14 respectively (p=0.024).

If the definition for success is set even higher, for example total pain relief, no patient in the placebo group obtained success. Four patients in the lignocaine group (17%) were pain free during periods after three pertubations compared to none in the placebo group (Fisher exact test, p=0.12) and the evaluated pain level on the VAS scale was only 0-4 (of 100).

It is interesting to note the long-term effect in ten patients in the PP population who improved ≥50% on the VAS scale. Nine of these were in the lignocaine group and the reduced pain level lasted up to one year after the first treatment. There is no apparent explanation of this long-term effect; however, macrophages have a long lifespan, ranging from months to years (183) and the effect might depend on permanent cellular effects (73, 116). Macrophages can switch their activation status from a pro-inflammatory to an anti-inflammatory state and the same macrophages can thereby participate in both the induction and the resolution of inflammation (184). In chronic inflammatory diseases, the inhibition of the apoptotic program promotes monocyte survival contributing to the accumulation of macrophages and the persistence of an inflammatory milieu (185).

The results of the pertubations with lignocaine or placebo were analysed in the PP and the ITT populations respectively since earlier studies had given indications that the effect on pain improved after repeated treatments (135). The reasons for not receiving the three treatments during three consecutive months were similar between groups and included bacterial vaginosis as well as patients on occasional vacation. Unfortunately, four patients did not fulfil the inclusion criteria VAS < 50 mm at inclusion and were therefore excluded from PP analysis. The chance of improvement >40 mm on the VAS scale is more or less eradicated if you start at a VAS below 50 mm.

No significant differences were seen in the success rate on the VAS scale between the lignocaine and the placebo groups three to nine months after the last treatment. This can be due to the small sample size, getting smaller for every time point.

Additional calculations presented in this thesis using the VAS scale as a continuous variable showed a significantly larger decrease on the VAS scale (with MWU test) in the lignocaine group after nine months compared to the placebo group. Even though a significant difference was seen, the clinical importance of this finding can be argued. The mean change in the lignocaine group was below the suggested MID and may therefore not be perceived by the patients and also the spread in the VAS change was large in both groups. These additional statistical analyses were exploratory and dichotomising the results in success/failure, using a cut off like the MID, is considered a better way to interpret the results in studies involving PRO. Also, the power analysis was based on proportions and there were no preliminary results considering changes on the VAS scale after lignocaine pertubations.

There were no significant differences between the groups when analysing the sum of scores from the categorical scales. The patients' change in function because of pain was equal with small improvements in both groups. Many patients had difficulty in graduating their function level on the categorical scales and continuous scales may be more sensitive to small changes compared to categorical scales.

Since no significant differences between treatment and placebo groups were seen in the ITT population, one could argue that an effect of lignocaine pertubations on pain could not be demonstrated. However, if using the MID on the VAS scale from the present sample there was a trend for larger improvement in the lignocaine group (p=0.10 for 50% and p=0.08 for 37mm) even in the ITT group. The significant effect on pain found in the PP population might be a coincidence but correspond to the effect on dysmenorrhea seen in the previous studies.

## 6.3 DISCUSSION PAPER III

Women with endometriosis have impaired HRQL compared to women without endometriosis and the QOL is related to the degree of pain. We hypothesized that pertubation with lignocaine could improve QOL in patients with endometriosis. A significantly larger improvement was found in the lignocaine group on the social support scale of the EHP-30 at six months. The four questions in the dimension social support measure the impact of endometriosis upon a woman´s social support network and reflect the patients feeling about her relations and her self confidence in social relations (Appendix 9.2)(167).

The baseline EHP-30 scores and demographics were comparable between the lignocaine and the placebo groups and the present baseline data on the EHP-30 is consistent with studies from other countries (153, 161, 165, 186-189). As in other populations, the highest score (i.e. worst quality of life) was found on the dimension control and powerlessness.

The lack of differences between the groups in 5/6 of the dimensions after six months was expected since the decrease in pain on the VAS scale was not significantly different between the groups after six months. After twelve months the sample size was probably too small to detect small differences between groups. Pertubation with lignocaine affects the degree of pain immediately after three pertubations i.e. approximately four months after the first treatment. The EHP-30 was not collected at this time-point since previous studies had shown long term effect. The EHP-30 questionnaire is extensive and in order to obtain good compliance, it was only collected at six and 12 months. One can speculate whether the effect on QOL had been more pronounced if the EHP-30 also had been collected after four month.

Although there was no difference in change in the pain intensity on the VAS scale after six months, a significant difference between the treatment and the placebo groups was seen for the dimension social support on the EHP-30. It has earlier been shown that HRQL depends on the degree of pain but other questionnaires were then used (19, 26). In the Short form-36 (SF-36) the social functioning score measures the impact of illness on the patient´s ability to continue with social activities whereas the social support dimension on the EHP-30 questionnaire measure the impact of endometriosis upon a woman´s social support network. The social support dimension on the EHP-30 questionnaire is probably related to the pain intensity even if the change in pain was similar between the groups after six months. Since the patients in the lignocaine group had significantly lower pain intensity two months before the second EHP-30 was filled out, it would be expected that the effect on social support was revealed thereafter and still depend on the pain intensity. Since the social support scale on the EHP-30 rather reflects the patients feeling about her relations, it would be expected that when she gets pain relief from the treatment, it might take some months to improve her self-confidence in social relations. Moreover, the effect seen on social support after six months might be a sustained effect from the hypothetic improved QOL two months earlier (when there was an effect on pain intensity).

Those who improved >50% on the VAS scale after four months had significantly better score on social support after six months ($p$=0.005) compared to those who did not improve. Other observations on the material show that many dimensions on the EHP-30 are related to the pain intensity and are affected when the pain level decreases more than 50% or if the maximum pain level reaches <20 mm on the VAS scale (unpublished data). Pertubation with lignocaine could have effects on quality of life independent on pain intensity even if the effect most probably is related to a lower pain level.

The power analysis was based on the change on the VAS-scale after 4 months and the EHP-30 questionnaire was a secondary outcome. Therefore the third study is probably underpowered and a larger sample size could be needed to fully answer the hypothesis of this study. Twelve parameters were evaluated (six dimensions at two time points) and the effect on social support might be a random finding i.e. due to mass significance.

It is difficult to interpret the results of PRO measures and the use of MID has been proposed to be helpful on this issue. The proportion of patients improving above the MID were

somewhat larger in the lignocaine group compared to the placebo group in 10 of 12 measurements but there were no significant differences except for social support after six months (Fisher´s exact test, p=0.036). An improvement above the MID is considered clinically significant and it is important to notice that the significant effect seen on social support could also be perceived by the patients.

## 6.4 DISCUSSION PAPER I AND III

Even in the placebo group there were patients that improved somewhat regarding pain and HRQL. This might be a placebo effect when participating in a clinical study. After four months, the mean effect of pertubation with placebo was 22.5 mm on the VAS scale, which is an effect of placebo in accordance with earlier studies (176, 177). Considerable effects on QOL were found in the placebo group after six months on the pain and the control and powerlessness scores. Placebo can have benefits in studies with continuous subjective and patient reported outcomes, for the treatment of pain and when a physical placebo intervention is used (179). A measurable placebo effect was therefore expected in the studies but is difficult to distinguish from biased reporting. The effect of placebo might improve any well-being in this population and be a confounder in a relatively small study.
In another RCT on patients with endometriosis, the placebo group demonstrated a significant improvement in the quality of life as measured by the SF-36 (26).

Some difficulties to include women in the study were expected since the preceding study by Edelstam *et al*. had to be terminated earlier due to difficulties of recruiting enough patients. Also in this study, the recruitment of patients was more difficult than expected. In total, 106 women were interested in participating, but 53 of them were not scheduled for a screening visit since they did not fulfil the inclusion criteria or were not interested after information had been given. Women with endometriosis and pain are hesitant to participate in a randomised study including placebo and therefore the present study was designed with unequal randomisation (4 treatment: 3 placebo) in order for more subjects to have a chance for treatment. Although previous studies indicated that lignocaine might be more effective, the study was designed to evaluate if treatment with lignocaine or placebo were the most effective. Every gynaecological examination is a potentially painful procedure for endometriosis patients and this, in combination with practical reasons such as geographic distance, were of importance for choosing not to participate. Many women did not fulfil inclusion criteria and the most common reason was a present wish for pregnancy. No further investigation on baseline characteristics for patients choosing not to participate has been conducted.

Women who have severe dysmenorrhea and side effects on medical treatments are more inclined to participate in a non-hormonal clinical trial. The fact that patients that have failed to improve on other treatments are attracted to clinical trials might constitute an attenuation biased group. The study also attracted women who had a wish for pregnancy since earlier studies had shown an effect on fertility. Even though they claimed no wish for pregnancy at inclusion, as many as six patients went through IVF after the three treatments had been given and were therefore lost to follow up. Also two women in lignocaine group who conceived naturally during the study were lost to follow up due to normal pregnancies. All children born after lignocaine pertubations were healthy.

Eleven women were withdrawn from the study because of pregnancy or need for other therapies and the drop-out rate was equal between the groups. Also the number of missing questionnaires was equal between groups. No analysis has been made on the patients that did not send in all questionnaires. A possible reason for not sending in the questionnaires could be disappointment due to lack of improvement after treatment. If that would be the reason, the results might be exaggerated since adding these missing questionnaires would dilute the effect in both lignocaine and placebo groups.
Since the present study is a double blind RCT, the risk for bias is considered to be low. The objective of randomisation is obtaining similar groups, however the small number of patients can be of more concern. The first and third studies have limitations due to the small sample size but the design contributes to the strength.

## 6.5 DISCUSSION PAPER II

Pertubation with lignocaine is safe. The serum levels of lignocaine following pertubation of 10 mg lignocaine hydrochloride are detectable but low. The highest level was 0.124µg/ml, which is about 80 times below the toxic level of 10µg/ml. The concentrations detected are consistent with other studies and correspond to the low dose pertubated (139). Study II support the theory that lignocaine pertubated through the fallopian tubes reaches the peritoneal cavity and diffuses through peritoneum into the blood circulation. The levels rose during the follow up time and the highest values were observed after 30 minutes. The major part of the pertubated fluid is thought to reach the peritoneal cavity but some lignocaine might also be absorbed by the endometrium or by the lining of the fallopian tubes during the pertubation process of approximately 5 minutes.

Lignocaine is a potent drug and a potential risk would be a high dosage of lignocaine in the central circulation. During the pertubation treatment the solution is infused into the uterine cavity and could possibly by accident be placed directly into a blood vessel. However, if the solution had accidently been infused in a vessel, the concentration in serum would have risen much faster. One patient had her highest level after 5 minutes but the level was very low (0.090 µg/ml).

The study has limitations due to the short follow up time and pharmacokinetics with $C_{max}$ and $T_{max}$ could therefore not be calculated. The sampling was not performed for longer time than 30 minutes after pertubation due to considerations for the patients. Earlier pharmacokinetic studies after intraperitoneal administration had indicated a $T_{max}$ ranging from 5 to 40 minutes and six of seven studies with plain lignocaine had a $T_{max}$ ranging between 5 and 30 minutes (139). The absorption of lignocaine was expected to be faster and the slower absorption registered might be due to the fact that no abdominal operation was carried out, which was the case in all of the reviewed studies. The $T_{max}$ for lignocaine ranges between 15 and 30 minutes after injection for dental anaesthesia as well as after a subcutaneous injection (138, 140). According to earlier studies the $T_{max}$ in our study is probably around 30 minutes and is unlikely to be above 40 minutes. Accordingly the $C_{max}$ could impossibly reach above 0.20 µg/ml after pertubation of 10 mg lignocaine.

The pertubation dosage of 10 mg was chosen as a safety precaution due to a minimal risk for depositing the substance directly into the circulation. An intravenous injection of 10 mg of lignocaine is known to be safe and the dosage would be far below the initial dosage for treatment of ventricular arrhythmia. An initial dose of 50-100 mg is then given intravenously (0.5-1.0

mg/kg bodyweight) as compared to the pertubated dose of 10 mg/70kg, approximately 0.14 mg/kg bodyweight.

Present data together with previous pharmacokinetic studies of lignocaine, confirm the hypothesis that pertubation with 10 mg lignocaine produce very low and therefore safe levels of lignocaine in serum.

The risk for pelvic infections secondary to repeated intra-uterine instrumentation and instillation are negligible. Only non-patent / post inflammatory tubes constitute a risk for infection (190). There are no reports on any infectious complications after repeated pertubation procedures such as Fallopian Sperm Perfusion for unexplained infertility and patent Fallopian tubes. LAs also possess antimicrobiological properties *in vitro* and *in vivo* (116) and patients treated with lignocaine are not more susceptible to infections (119).

## 6.6 DISCUSSION PAPER IV

The EHP-30 questionnaire used was a Swedish translation used by Pharmacia UpJohn 2001, which was available at the research unit at Danderyd hospital. The items in Swedish were compared to the English original version and seemed to be correctly translated with no obvious linguistical or conceptual differences between the original and translated versions. In 2010, a new translation to Swedish was performed by Isis Innovation, the owner of the EHP-30 questionnaire. When comparing the used translation with the translation by Isis Innovation, only minor linguistic changes between the translations were found (Appendix 9.2 and 9.3).

Adaption of a questionnaire to other languages includes not only translation of items and response scales (152). The psychometric properties of the questionnaire may differ between different cultures and ideally a questionnaire should be evaluated and adapted in each population. It is important that the concepts assessed by each item should be as identical as possible, the aggregation of items should result in the same constructs and the metric of scales should be similar.

The EHP-30 is recommended as an outcome measure in clinical trials involving patients with endometriosis and pain and has been shown to be more responsive to change than the general questionnaire SF-36 (153). Even if the EHP-30 has not yet been validated in Swedish, it was considered the best available option since it is the only QOL scale that has been validated for use in women with endometriosis (74). The cultures in Sweden and Great Britain are not very different and the psychometric properties of the EHP-30 questionnaire are most probably similar in the Swedish and the English version. The Dutch version of the EHP-30 has been showed to be reliable, valid and responsive to change (165, 187). Most translation guidelines assume that constructs are relevant and equivalent across cultures (152, 191). However, a validation of the Swedish version preceding the clinical trial would have been preferable. The core questionnaire and the sexual intercourse questions were available in Swedish at the research unit and were therefore used. Using the whole questionnaire might affect compliance and was not an option. The score concerning sexual intercourse were included since this is a frequent problem for women with endometriosis (25, 145). The general details and the general health questions in the EHP-30 questionnaire were not included but similar data were gathered in the CRF (Case record form).

The EHP-30 questionnaire translated to Swedish seems to be acceptable, understandable and applicable. Data completeness for the core scales were high for all dimensions (89-99%) and only a few questions remained unanswered. The sexual intercourse scale was the one with most missing items and not all women were sexually active at the different time points. About half of the women that did not have sex avoided intercourse because of pain and the other half lacked sexual partner at that specific time point. Due to pelvic pain and subsequent relation problems, absence or infrequent intercourse is common among women with endometriosis. The relatively low dropout rate was probably partly due to a limited number of modular items. The sample in this clinical trial seems to have normal distributions on all the different scores with few patients at extreme values.

It is important that the QOL questionnaires used in clinical trials are responsive to change and it is recommended to confirm responsiveness across multiple samples (150). Also, to be able to trust the QOL result, it was important to demonstrate responsiveness of the EHP-30 in the study population. The social support scale on the EHP-30 questionnaire failed to demonstrate responsiveness in a sample of 40 women in the U.K. (153) but did show responsiveness in a larger sample of 228 women in the Netherlands (165). In our material, the EHP-30 questionnaire seems to be highly responsive to change for all scales on the core questionnaire. The study sample was small but equal to the sample size used by Jones et al. when initially evaluating the responsiveness of the questionnaire (153).

In the improved group there were significant changes on all core scales on the EHP-30 questionnaire and the effect sizes were moderate to large, indicating that the EHP-30 is highly responsive to change. Similar to earlier studies, the most responsive scales were pain and control and powerlessness (153, 165). No significant changes in any dimension on the EHP-30 were seen in the stable group and the effect sizes were small indicating little change in health status.
It is vital that a HRQL questionnaire to be used in a clinical trial can discriminate between improved and non-improved patients. Data presented in this thesis indicate that the EHP-30 questionnaire is well suited for this purpose. Significant differences were found between the improved and the stable groups for the change in EHP-30 scores for pain, control and powerlessness and emotional well-being whereas a tendency for significance was seen for social support and self image.

The HRQL is related to pain intensity in the present study. A correlation between the mean change on the EHP-30 scores and the patients own estimation of change in pain intensity was found. The fact that pain intensity is correlated with QOL is in concordance with earlier studies (19, 26). We used the patients´ estimation of change in pain intensity as an anchor to evaluate responsiveness since pain has relationship with HRQL (150).

In the small group of patients that evaluated their pain to be the same (n=6) the effect sizes for pain and control and powerlessness were moderate but there were no significant changes (paired t-test) in the different EHP-30 scores. Thus, some of the scales displayed responsiveness in the small group that felt the same but for the other dimensions the effect sizes were small, indicating small changes in quality of life for patients reporting themselves to feel the same. Although some of the scales were responsive for the stable group, in all cases they were less responsive than in

the improved group. Similar results have been obtained when Colwell´s QOL questionnaire was evaluated, displaying responsiveness on some of the scales in the stable group (145). Even in the Dutch population, small improvements were seen in the non-change group (165). The reason for improved QOL, even if the pain intensity was estimated to be the same, might be a placebo effect when participating in a clinical study or an effect of reporting bias (178, 179, 192).

The EHP-30 questionnaire does not seem to be as responsive for deterioration as it is for improvement. For patients that felt worse in pain intensity, the effect sizes were much smaller than in the improved group and the changes on the different EHP-30 scores were not significant. There is evidence for asymmetry in worsening and improvement in patient reported outcomes (150). The asymmetry in our data is in accordance with Colwell´s QOL questionnaire, where responsiveness was found to be moderate to high for the patients who improved but low to moderate in the impaired group (145). Also in a Dutch study evaluating the responsiveness of the EHP-30 questionnaire, the changes in scores for those who deteriorated were smaller than the changes in the improved group (165).

Only the EHP-30 questionnaires collected after six months were used in the responsiveness analysis. This was due to a smaller sample size at twelve months and the fact that a longer time had passed since inclusion, making the patients own evaluation of change in pain intensity more unreliable.

The MID may vary by population and context and no level for MID will be valid for all study applications involving a PRO instrument (150, 171). In the study population, the MID levels on the EHP-30 questionnaire were higher for emotional well-being and social support but lower for control and powerlessness, pain and self confidence compared to the levels in Jones´ study, based on 40 patients (153). The MIDs in the present data were larger for all dimensions in comparison with the study by van de Burgt *et al*. (165). The MID in social support was -13 compared to -10 in the Dutch study and +1.7 in the British study supporting the thesis that the EHP-30 questionnaire is sensitive to change even for the dimension social support.

To be able to compare our results with the study by Jones *et al*. the same parametric tests were used (153). Mostly parametric tests are used when evaluating responsiveness (147). However, in this small study in which an ordinal scale was used it would have been more appropriate with non-parametric tests even though the data seemed to be normally distributed. The results in this thesis have been complemented with non-parametric tests and similar results were obtained.

Data from the fourth study show that the EHP-30 is sensitive to change on all dimensions on the core questionnaire and can detect differences in health related quality of life at a level that is important and detectable for the patients.

## 6.7 DISCUSSION PAPER V

It was hypothesized that lignocaine could attenuate the gene expression and the release of the pro inflammatory cytokines IL-6, IL-8 and MCP-1 *in vitro.* The main finding was a significant decrease in both the gene expression and the protein secretion of IL-8 in endometriotic stromal cells after incubation with lignocaine 0.1 mg/ml for 24h.

Lignocaine has been shown to decrease cytokine release both *in vitro* and *in vivo* but it is not known whether this is due to a reduced gene expression and synthesis of the cytokine or decreased cellular secretion (122). The present data is in accordance with a study on epithelial cells, in which both the secretion and the transcription of IL-8 was reduced by treatment with lignocaine (118). A tendency for both lower gene expression and protein secretion of IL-6 was found in macrophages from endometriosis patients treated with lignocaine. In contrast, for the healthy macrophages the gene expressions were lower for all tested cytokines whereas no significant differences on protein levels were found. The mechanism for the effect of lignocaine is thereby still unknown and may differ between different cell types.

Lignocaine 0.1mg/ml correspond to a concentration of 0.43 mM since the molecular weight of lignocaine is 234 Da. Lignocaine at higher concentrations than 0.5 mg/ml, had effect on both proliferation and viability and could therefore be considered to be cell toxic. This was also seen after RNA preparation where the RNA concentration was considerably lower after incubation with lignocaine 1.0 mg/ml. Therefore the incubations with lignocaine 1.0 mg/ml were excluded from further analysis.
It can be presumed that lignocaine 0.1 mg/ml is not toxic to cells *in vitro* even though a significant decrease in viability was seen at 0.05 and 0.1 mg/ml compared to the control after 24 h. The proliferation at the same concentration and time-point was not affected and the spread between samples was large. The effect of lignocaine on cell viability has been evaluated in other studies. A lignocaine dosage of 0.4 mg/ml for 24 h did not affect the viability of adipose stem cells in culture compared to controls whereas concentrations of 0.8 mg/ml and 1.6 mg/ml showed a significantly increased cytotoxicity (193). Other studies have shown no effect on the viability of epithelial cell after exposure to lignocaine 0.23 mg/ml for 24h (118) or of endothelial cells after exposure to lignocaine 0.5 mg/ml for 4h (131).

The dosage of 0.1 mg/ml used corresponds to the probable situation *in vivo* after pertubation with 10 mg lignocaine. The peritoneal fluid dilutes the dosage of 10 mg in the clinical studies and corresponds to an approximated intraperitoneal lignocaine-concentration of 0.1-0.3 mg/ml. Other *in vitro* studies evaluating the effect of lignocaine on cytokine levels used concentrations ranging from 0.011-0.7 mg/ml and the cells were incubated with lignocaine for 4h-24h (118, 129, 131). A maximal effect on cytokine secretion was seen at a lignocaine concentration of 0.23 mg/ml when epithelial cells were cultured for 24h (118).

IL-8 is thought to play a role in the pathogenesis and maintenance of endometriosis since it may act as an autocrine growth factor in the endometrium (29). Lower concentrations of IL-8 in the PF could have beneficial effects on both the symptoms and the progression of endometriosis. The lower gene expression and secretion of IL-8 in endometriotic stromal

cells in our study can thus be part of an explanation for the clinical effect seen on pain and fertility after pertubation of lignocaine *in vivo*.

The macrophages from women with endometriosis had a significantly higher production of IL-8 compared to macrophages from healthy women, which is in concordance with earlier studies. Macrophages from patients with endometriosis are more activated compared to macrophages from healthy women (28). The increased concentration of IL-8 in the PF of patients with endometriosis has been suggested to be derived from peritoneal macrophages (51, 194). The IL-6 response in peritoneal macrophages and endometrial stromal cells have been found to be dysregulated in patients with endometriosis (195).

Macrophages from patients with endometriosis and healthy controls reacted different on treatment with lignocaine. All cell cultures from healthy women (n=5) showed a decreased gene expression of all the tested pro-inflammatory cytokines after incubation with lignocaine compared to untreated cells from the same healthy women. In contrast, lignocaine had diverging effects on gene expression in the macrophages from patients with endometriosis since three of five samples showed increased gene expression of one (n=2) or two (n=1) cytokines after lignocaine treatment.
These results indicate that some patients with endometriosis may have macrophages that are altered and do not react normally. This could be an explanation for the fact that the clinical effect on pain after pertubation with lignocaine differed between patients.

After 48 h incubation with lignocaine, no differences were seen in gene expression or protein levels of MCP-1, IL-6 and IL-8 in endometriotic stromal cells. This was somewhat surprising since a pilot study on only one sample showed lower concentrations of MCP-1 after both 24 h and 48 h and we had expected the same trend in this larger study of 7 patients. As with the macrophages, also the endometriotic stromal cells might differ between patients with endometriosis and subsequently some patients might benefit from lignocaine pertubations whereas others might not. The diverging reactions in stromal cells after treatment with lignocaine illustrate the importance of larger studies with several samples. The sample size of seven can be seen as strength in this *in vitro* study but even more patients would have been preferable.

There were economic reasons for choosing only three cytokines in the *in vitro* study. IL-6, IL-8 and MCP-1 was chosen since their levels in peritoneal fluid are related to the severity of endometriosis, since they are all believed to be involved in the pathogenesis of endometriosis and since they are all secreted by both macrophages and ectopic endometrial cells (stromal and epithelial cells).

## 6.8 IMPLICATIONS

Pertubation with lignocaine can be developed to be a new non-hormonal treatment option for endometriosis-related menstrual pain in patients with minimal to moderate endometriosis. The data presented in this thesis is not strong enough to prove the effect of lignocaine pertubations on endometriosis associated pain but can act as a platform for future studies. The study population represents a small and selected part of the whole endometriosis population and might not be representative for all patients with endometriosis. However, it

represent patients that have a need for non-hormonal therapies due to side effects, patients with a wish for pregnancy and patients who have failed to improve on other treatments. Since these patients are common in clinical endometriosis practice, at least some of the results can probably be transformed on the whole endometriosis population.

## 6.9 FUTURE PERSPECTIVES

Well-designed clinical and cellular studies are needed to support the potent anti-inflammatory effects of LAs in various clinical conditions and to form a platform for future treatments of inflammation (115, 116).

The optimal dosage and therapy intervals for the clinical effect seen on fertility and pain are not known. Studies are in progress to further evaluate the effect on pain and to assess the optimal dosage and therapy intervals. The pharmacokinetic study gives indications that it would be possible to try a higher dosage with preserved safety. With a higher dosage it could be possible to give fewer treatments or to have longer therapy intervals.

In a forthcoming phase III study, the effect on pain, quality of life and fertility could be evaluated in a larger sample. The number of exclusion criteria could be reduced to make the inclusion of patients easier. For example, patients with a present wish for pregnancy and women on continuous OC due to pelvic pain could be included. This would also reduce the risk of bias. After the last pertubation, the HRQL should be evaluated during the subsequent period and maybe more frequently thereafter. There is a need for an additional pharmacokinetic study with longer follow up and maybe different dosages.
It is obvious that some patients benefit from lignocaine pertubations whereas others have no effect at all. This could also be seen at a cellular level. A larger study could help us identify those with highest chance for improvement. Identifiable factors that might have an effect on outcome are duration of endometriosis, fertility, age, smoking, concomitant depression, baseline quality of life, baseline pain level during and between periods and effects of previous treatments.

In more advanced endometriosis, one could evaluate the effect of lignocaine on pain if deposed in the peritoneal cavity during diagnostic or therapeutic laparoscopy.

Pertubation with lignocaine probably affects the endometrium during its passage and have effect on fertility which would be interesting to investigate at a pathohistological level. Maybe the sensory nerve fibres or levels of cytokines in the endometrium are affected by lignocaine, leading to a higher implantation rate.
The mechanism of lignocaine on peritoneal macrophages and ectopic endometrium *in vitro* needs to be further investigated. In cellular studies it would be interesting to investigate other dosages of lignocaine, other treatment durations, other cytokines and also other types of cells i.e. ectopic epithelial cells. A somewhat higher concentration of lignocaine may give other results since one study has shown maximal effect on cytokine secretion at a lignocaine concentration of 0.23 mg/ml (118).

# 7 CONCLUSIONS

Pertubation with lignocaine can relieve pain in some patients with endometriosis and might be developed to be a new non-hormonal treatment option for endometriosis-related pain in patients with minimal to moderate endometriosis. During pertubation treatments, ovulatory cycles can be maintained and fertility preserved. The treatment is easy to perform with good safety in an outpatient setting.

Lignocaine pertubated through the fallopian tubes reaches the peritoneal cavity and diffuses through the peritoneum into the blood circulation. The serum levels of lignocaine following pertubation of 10 mg lignocaine hydrochloride are detectable but low. Pertubation with lignocaine is safe, well tolerated and have no adverse events related to lignocaine.

Pertubations with lignocaine might improve the social support dimension of quality of life in patients with endometriosis. The effect is probably due to decreased pain levels during the preceding months. To further evaluate the effect of lignocaine pertubations on quality of life, a larger study with more frequent follow up time points is required.

The EHP-30 is responsive to improvement on all dimensions on the core questionnaire. The Swedish version of the EHP-30 questionnaires used in the present thesis seems to be acceptable, understandable and applicable in our Swedish material. Quality of life is related to the degree of pain.

Lignocaine inhibit the gene expression and secretion of IL-8 in endometriotic stromal cells. Further, lignocaine inhibit the gene expression of IL-6, IL-8 and MCP-1 in healthy macrophages *in vitro*. Macrophages from women with endometriosis might be dysregulated. The data presented in this thesis indicate that due to differences at cellular level, nearly half of the endometriosis population may benefit from lignocaine pertubations.

Thus, the findings of this thesis support the hypothesis that pertubation with lignocaine can relieve pain in some patients with endometriosis and that the treatment could also have an effect on Quality of Life. The in vitro study demonstrate that lignocaine have cellular effects in low concentrations which is promising for the development of lignocaine-pertubations as a new non-hormonal treatment for pain due to endometriosis.

# 8 POPULÄRVETENSKAPLIG SAMMANFATTNING

**Bakgrund endometrios**
Endometrios är en relativt vanlig sjukdom som drabbar 6-10 % av kvinnor i fertil ålder och upp till 50 % av kvinnor med mensvärk och infertilitet (5-8). Vid endometrios förekommer livmoderslemhinna på andra ställen än i livmodern, oftast på bukhinnan i nedre delen av buken och på äggstockarna . Sammanväxningar kring äggstockar och äggledare kan uppstå vilket kan skada anatomin och försämra passagen i äggledarna (3).
De vanligaste symtomen vid endometrios är mensvärk, smärta i nedre delen av buken mellan menstruationerna och nedsatt fertilitet (5-8). Kvinnor med endometrios har oftare än andra kvinnor depression och ångest vilket är relaterat till graden av smärta (19-21). Även så kallad hälsorelaterad livskvalitet är försämrad hos kvinnor med endometrios (19, 23) beroende på långvarig smärta (19, 24, 26) och nedsatt fertilitet (27). 30-50% av kvinnor med endometrios är infertila (3, 11, 28) och av infertila kvinnor har 25-40% endometrios (11, 16).
Diagnosen kan ställas vid titthålsoperation då de typiska härdarna kan ses (9, 10).

Endometrios uppstår enligt rådande hypotes, genom att menstruationsblod med fragment av livmoderslemhinna går bakvägen genom äggledarna ut i bukhålan i samband med menstruation. Riskfaktorer innefattar riklig eller långvarig mens och missbildningar som försvårar det normala avflödet för menstruationen (5, 28, 37-39). Det finns också en stark ärftlig komponent (6, 31, 32). Man har hittat förändringar i immunsystemet hos kvinnor med endometrios, vilket kan ha betydelse för sjukdomens utveckling och fortskridande (38, 42). Förändringarna kan påverka slemhinnefragmentens förmåga att fästa och överleva på bukhinnan (29, 32) och det uppstår en kronisk inflammation i bukhålan hos kvinnor med endometrios. Bukvätskan innehåller fler immunologiska celler och inflammatoriska substanser jämfört med bukvätskan hos friska (28, 42, 45). Inflammationen ger upphov till symtom såsom smärta, infertilitet och sammanväxningar (28, 30, 42).

Det finns ingen permanent bot för endometrios utan tillgängliga behandlingar syftar till att lindra symtomen (16). Smärtstillande tabletter och hormonella behandlingar som slår ut mensen (t ex p-piller) är effektiva liksom kirurgisk behandling då man förstör härdarna och tar bort endometrioscystor. Risken är dock stor att besvären återkommer efter att behandlingen avslutats (8, 9). De hormonella behandlingarna har biverkningar och fungerar som preventivmedel genom at hämma ägglossning.

**Bakgrund lidokain**
Lidokain är ett lokalbedövningsmedel som först framställdes av den svenske kemisten Nils Löfgren 1943. Lidokain i höga koncentrationer blockerar nervimpulser och impulser mellan hjärtmuskelceller och används som lokalbedövningsmedel och vid hjärtrytmrubbningar (115). De senaste två decennierna har man upptäckt att lokalbedövningsmedel i låga koncentrationer har anti-inflammatoriska egenskaper (115, 118, 122, 133). Om lidokain sköljs genom livmoderhålan och genom äggledarna ut i fri bukhåla (pertubation), så ökar chanserna till graviditet (134, 135). Ett bifynd vid dessa fertilitetsstudier var minskad mensvärk hos de kvinnor som inte blev gravida (137). Höga doser av lidokain kan ge bieffekter (138, 139) och uppstår vid koncentrationer i blod kring 3-5 µg/ml (116, 138). Nivåer överstigande 20 µg/ml kan orsaka hjärtstopp (138, 140).

**Bakgrund livskvalitet**
Hälsorelaterad livskvalitet mäter fysiska, emotionella och sociala aspekter associerade med en viss sjukdom eller dess behandling med hjälp av särskilda formulär (145, 146). Ett frågeformulär som skall användas vid kliniska studier måste kunna registrera förändringar (149, 150). EHP-30 (Endometriosis Health Profile-30) är det enda formulär för endometrios som är validerat och det har visat sig vara känsligt för förändring på alla skalor utom en som heter social support eller socialt stöd (146, 153, 161).

**Mål med studierna**
Studierna i denna avhandling syftar till att utvärdera effekten på smärta och livskvalitet hos kvinnor med endometrios efter pertubation (genomsköljning) med lidokain. Nivåerna av lidokain i blodet efter behandling mättes för att utvärdera metodens säkerhet. Vidare utvärderades om EHP-30 var känsligt för förändring i det aktuella svenska patientmaterialet (150).
En förklaring till kliniska effekter av lidokain på smärta och fertilitet har studerats på cellnivå.

**Metoder**
Studierna har godkänts av Regionala etikprövningsnämnden i Stockholm och av Läkemedelsverket.
I studie I-IV deltog 42 kvinnor av vilka 24 randomiserades till pertubationsbehandling med lidokain och 18 till behandling med placebo. Deltagarna hade menssmärta och endometrios som diagnosticerats med titthålsoperation. Tre behandlingar gavs under tre menscykler och behandlingseffekten utvärderades med enkäter i upp till ett år efter påbörjad behandling. Enkäterna utvärderade smärta och livskvalitet (EHP-30). Blodprover togs före och upp till 30 minuter efter behandling för att mäta halterna av lidokain. Med hjälp av svaren på enkäterna utvärderades EHP-30s känslighet för förändring.
I studie V togs vävnadsprov från bukvätska och äggstocks-cystor från 13 kvinnor i samband med planerad operation. Kvinnorna hade endometrios eller var friska. Celler från bukvätska och från endometrioscystor odlades med eller utan tillsats av lidokain. Produktionen och utsöndringen av tre inflammatoriska substanser mättes och jämfördes mellan celler som odlats med eller utan lidokain.

**Resultat**
Av de kvinnor som behandlats med lidokain var signifikant fler förbättrade avseende smärtan efter sista behandlingen (tio av 24), jämfört med de kvinnor som behandlats med placebo (tre av 18). Hos de som förbättrades, kvarstod effekten hos hälften i upp till nio månader efter tredje behandlingen. Drygt två månader efter sista behandlingen hade kvinnorna som behandlats med lidokain en större förändring på skalan socialt stöd på EHP-30 jämfört med de som behandlats med placebo (-18.8 respektive -6.3). Inga skillnader mellan grupperna kunde påvisas efter drygt nio månader.
Nivåerna av lidokain i blodet efter behandling var mycket låga och den högsta nivån var 0,124 µg/ml vilket uppmättes efter 30 minuter. Inga biverkningar beroende på lidokain sågs och behandlingen tolererades väl.
EHP-30 registrerade förändringar på alla skalor i det aktuella patientmaterialet. De som kände sig bättre avseende smärta hade större förändringar på enkäten jämfört med de som var oförändrade eller försämrade.
Cellerna från cystorna som odlades med lidokain hade ett lägre genuttryck och utsöndrade lägre halter av den inflammatoriska substansen IL-8 jämfört med cellerna som odlats utan lidokain (4.20 pg/µg RNA protein med lidokain jfr med 5.95 utan). Tillsats av lidokain hämmade genuttrycket för de studerade inflammatoriska substanserna (IL-6, IL-8, MCP-1) i alla cellinjer från bukvätskan hos friska kvinnor. Celler från bukvätskan hos kvinnor med endometrios reagerade mer divergerande med såväl minskat som ökat genuttryck.

**Konklusion**
Pertubationbehandling med lidokain kan minska smärtan och förbättra livskvaliteten hos kvinnor med endometrios. Materialet var dock för litet för att säkra slutsatser skall kunna dras. Behandlingen är säker och utan biverkningar och mycket låga halter av lidokain kan ses i blodet. Enkäten som användes för att utvärdera livskvalitet var känslig för förändring i den studerade patientgruppen, och resultaten således pålitliga. Lidokain kan påverka genuttryck och utsöndring av inflammatoriska substanser i cellodlingar med celler från endometrioscystor. Celler från bukvätska hos kvinnor med endometrios reagerar inte alltid som celler från friska kvinnor. Detta kan vara en del i förklaringen till att inte alla kvinnor förbättrades av pertubationsbehandlingen. Ytterligare studier behövs för att bekräfta effekten av lidokain på smärta och livskvalitet vid endometrios. Studien av koncentrationen i blodet, visar att man utan risk kan öka lidokaindosen något för att om möjligt förbättra effekten.

# 9 APPENDIX

## 9.1 PAIN QUESTIONNAIRE

### Smärtdagbok

Smärtdagboken skall ifyllas i samband med menstruationen, nämligen veckan innan mens, under mens och veckan efter mens. För vart och ett av de uppräknade symtomen skall du, från tabellen nedan, välja den **kod** som bäst stämmer överens med den upplevda intensiteten. För varje kategori skall du ange den svåraste smärtan du haft den dagen. Varje intensitet är beskriven längre ned på sidan.
Om du skulle glömma att fylla i någon dag eller inte kan komma ihåg hur du känt dig, ber vi dig att dra ett streck över den aktuella rutan.

| Symtom | Veckan före mens | Mens 1-a dag | Mens 3-e dag | Mens 5-e dag | Veckan efter mens |
|---|---|---|---|---|---|
| Datum (dag/månad) | | | | | |
| **Smärta under menstruation** | | | | | |
| **Magsmärta** | | | | | |
| **Djup smärta under samlag** | | | | | |
| **Använde du någon smärtstillande medicin?** | Ja | Ja | Ja | Ja | Ja |
| | Nej | Nej | Nej | Nej | Nej |

**Beskrivning av symtom**

| Symtom | Svårighetsgrad | Kod | Beskrivning |
|---|---|---|---|
| Smärta under menstruation | Inte relevant | 0 | Ingen menstruation idag |
| | Ingen | 1 | Ingen smärta |
| | Mild | 2 | Viss förlust av arbetskapacitet |
| | Måttlig | 3 | Sängliggande delar av dagen, inte arbetsför |
| | Svår | 4 | Sängliggande större delen av dagen, inte arbetsför |
| Magsmärta (smärta förutom under mens) | Inte relevant | 0 | Menstruation idag |
| | Ingen | 1 | Ingen magsmärta |
| | Mild | 2 | Tillfällig smärta i magen |
| | Måttlig | 3 | Påtaglig smärta större delen av dagen |
| | Svår | 4 | Ihållande smärta som kräver starka smärtstillande medel |
| Djup smärta under samlag | Inte relevant | 0 | Inget samlag av olika andra själ |
| | Ingen | 1 | Ingen smärta |
| | Mild | 2 | Acceptabelt obehag |
| | Måttlig | 3 | Samlaget avbrutet pga. smärta |
| | Svår | 4 | Undvek samlag pga. smärta |

**Smärtskala(VAS)**

Du skall här ange den svåraste smärta du upplevt under de senaste tre veckorna d vs under tidsperioden ovan. (VAS-skala, sätt ett streck på linjen nedan)

Ingen smärta _____ Värsta tänkbara smärta

## Under ovanstående treveckorsperiod...

**Sjukskrivning**
Hur många dagar har du behövt vara sjukskriven pga. dina smärtor?(Sätt gärna ett kryss el streck/dag)

_____

**Smärtstillande läkemedel**
Hur mycket smärtstillande mediciner har du behövt senaste **tre veckorna?** Ange sort, styrka och ungefärligt antal. (Sätt kryss/streck eller ange antal)

| **Exempel på smärtstillande mediciner** | **styrka** | **<5** | **5-10** | **10-15** | **>15** | **Mer?** |
|---|---|---|---|---|---|---|
| Paracetamol (Alvedon, Panodil) | | | | | | |
| NSAID ( Ipren, Voltaren, Naproxen, Orudis osv) | | | | | | |
| Kodein ( Citodon, Panacod, Treo Comp) | | | | | | |
| Dextropropoxifen(Dexofen, Doloxene, Distagesic | | | | | | |
| Tramadol( Tradolan, Nobligan, Tiparol | | | | | | |
| Annat? | | | | | | |

## Förändring?

Upplever du att värken förändrats efter ditt deltagande i studien? Fyll i tabellen genom att ange kod enligt tabellen bredvid.

| **Symtom** | **förändringskod** |
|---|---|
| Menssmärta | |
| Magsmärta utan mens | |
| Djup smärta vid samlag | |

| **Kod** | **Beskrivning** |
|---|---|
| 0 | Nej, värken är oförändrad |
| 1 | Ja, bättre men har fortfarande besvär |
| 2 | Ja, helt smärtfri |
| 3 | Ja, jag har lite mer ont än innan |
| 4 | Ja, jag har mycket mer ont än innan |

Har ditt behov av smärtstillande läkemedel förändrats efter ditt deltagande i studien? (sätt X)

| Kod | **Beskrivning** | **Sätt kryss här nedanför!** |
|---|---|---|
| 0 | Nej, ingen förändring | |
| 1 | Ja, mindre behov | |
| 2 | Ja, större behov av smärtstillande | |

## Biverkningar?

Har du upplevt någon biverkan sedan du gick med i studien? Annat? Skriv nedan!

_____

## Vem är du?

Sista fyra siffrorna i p-nr:_____ Initialer:_____
Datum:_____

## 9.2 USED VERSION EHP-30

**EHP 30** *(Page 1 of 2)*

Patient initials: _ _ _ Patient number: _ _ _

☐ *Page not done*

| I följande frågor ber vi dig bedöma hur endometrios har påverkat ditt liv de 4 senaste veckorna. För varje fråga sätter du ett "x" i rutan vid det svar som bäst motsvarar dina känslor och erfarenheter. | | | | | |
|---|---|---|---|---|---|
| **Hur ofta de senaste 4 veckorna har du på grund av endometrios...** | **ALDRIG** | **SÄLLAN** | **IBLAND** | **OFTA** | **ALLTID** |
| 1. varit oförmögen att delta i sociala aktiviteter på grund av smärtan? | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 2. varit oförmögen att utföra sysslor i hemmet på grund av smärtan? | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 3. haft svårt att stå på grund av smärtan? | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 4. haft svårt att sitta på grund av smärtan? | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 5. haft svårt att gå på grund av smärtan? | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 6. haft svårt att motionera eller utöva de fritidsaktiviteter som du skulle vilja? | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 7. förlorat matlusten och/eller inte kunnat äta på grund av smärtan? | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 8. haft sömnsvårigheter på grund av smärtan? | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 9. varit tvungen att gå till sängs/ligga ner på grund av smärtan? | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 10. varit oförmögen att göra de saker som du velat på grund av smärtan? | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 11. känt dig oförmögen att stå ut med smärtan? | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 12. mått allmänt dåligt? | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 13. känt dig frustrerad eftersom symtomen inte förbättras? | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 14. känt dig frustrerad för att du inte har någon kontroll över symptomen? | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 15. känt att det varit omöjligt att glömma bort symptomen? | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 16. känt det som om symptomen styr ditt liv? | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 17. känt att symptomen håller på att ta över ditt liv? | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 18. känt dig deprimerad? | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 19. känt dig gråtfärdig/gråtmild? | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |

Kontrollera att du har satt ett "X" i en av rutorna vid varje fråga, innan du går vidare till nästa sida.

Patient's Initials: _____

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ORIGINAL

Patient initials: _ _ _ Patient number: _ _ _

☐ *Page not done*

| Hur ofta de senaste 4 veckorna har du på grund av endometrios... | ALDRIG | SÄLLAN | IBLAND | OFTA | ALLTID |
|---|---|---|---|---|---|
| 20. känt dig eländig? | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 21. haft humörsvängningar? | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 22. varit på dåligt humör eller retlig? | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 23. känt dig våldsam eller aggresiv? | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 24. känt dig oförmögen att tala om för andra hur du känner dig? | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 25. känt att andra inte förstår vad du går igenom? | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 26. känt det som om andra tycker att du klagar för mycket? | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 27. känt dig ensam? | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 28. känt dig frustrerad över att inte alltid kunna ha på dig de kläder som du skulle vilja? | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 29. känt att ditt utseende har påverkats? | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 30. saknat självförtroende? | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |

**Del 2**

**Dessa frågor gäller hur endometrios har påverkat dina sexuella förhållanden de senaste 4 veckorna.**

| Hur ofta de senaste 4 veckorna har du på grund av endometrios... | ALDRIG | SÄLLAN | IBLAND | OFTA | ALLTID |
|---|---|---|---|---|---|
| 1. upplevt smärta under eller efter samlag? *Om ej tillämpligt, sätt ett "X" här* ☐0 | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 2. känt oro för att ha samlag på grund av smärtan? *Om ej tillämpligt, sätt ett "X" här* ☐0 | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 3. undvikit samlag på grund av smärtan? *Om ej tillämpligt, sätt ett "X" här* ☐0 | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 4. haft skuldkänslor för att du inte velat ha samlag? *Om ej tillämpligt, sätt ett "X" här* ☐0 | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 5. känt dig frustrerad över att inte kunna njuta av samlag? *Om ej tillämpligt, sätt ett "X" här* ☐0 | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |

**Kontrollera att du har satt ett "X" i en av rutorna vid varje fråga, innan du går vidare till nästa sida.**

Patient's Initials: _ _ _

## 9.3 EHP-30 TRANSLATION BY ISIS INNOVATION

# EHP-30

Har du besvarat EHP-30 förut? ☐ Ja ☐ Nej

| HUR OFTA UNDER DE SENASTE 4 VECKORNA HAR DU PÅ GRUND AV DIN ENDOMETRIOS ... | Aldrig | Sällan | Ibland | Ofta | Alltid |
|---|---|---|---|---|---|
| 1. Inte kunnat vara med på sociala evenemang på grund av smärtan? | ☐ | ☐ | ☐ | ☐ | ☐ |
| 2. Inte kunnat utföra sysslor i hemmet på grund av smärtan? | ☐ | ☐ | ☐ | ☐ | ☐ |
| 3. Haft svårt att stå på grund av smärtan? | ☐ | ☐ | ☐ | ☐ | ☐ |
| 4. Haft svårt att sitta på grund av smärtan? | ☐ | ☐ | ☐ | ☐ | ☐ |
| 5. Haft svårt att gå på grund av smärtan? | ☐ | ☐ | ☐ | ☐ | ☐ |
| 6. Haft svårt att motionera eller utöva de fritidsaktiviteter du velat göra på grund av smärtan? | ☐ | ☐ | ☐ | ☐ | ☐ |
| 7. Tappat aptiten och/eller inte kunnat äta på grund av smärtan? | ☐ | ☐ | ☐ | ☐ | ☐ |

Var vänlig kontrollera att du har kryssat i ***en ruta för varje fråga*** innan du går vidare till nästa sida

| HUR OFTA UNDER DE SENASTE 4 VECKORNA HAR DU PÅ GRUND AV DIN ENDOMETRIOS ..... | Aldrig | Sällan | Ibland | Ofta | Alltid |
|---|---|---|---|---|---|
| 8. Inte kunnat sova ordentligt på grund av smärtan? | ☐ | ☐ | ☐ | ☐ | ☐ |
| 9. Varit tvungen att gå till sängs/ligga ner på grund av smärtan? | ☐ | ☐ | ☐ | ☐ | ☐ |
| 10. Inte kunnat göra de saker du velat göra på grund av smärtan? | ☐ | ☐ | ☐ | ☐ | ☐ |
| 11. Känt att du inte kunnat hantera smärtan? | ☐ | ☐ | ☐ | ☐ | ☐ |
| 12. Känt dig allmänt krasslig? | ☐ | ☐ | ☐ | ☐ | ☐ |
| 13. Känt dig frustrerad över att dina symtom inte blir bättre? | ☐ | ☐ | ☐ | ☐ | ☐ |
| 14. Känt dig frustrerad över att du inte kan kontrollera dina symtom? | ☐ | ☐ | ☐ | ☐ | ☐ |

Var vänlig kontrollera att du har kryssat i ***en ruta för varje fråga*** innan du går vidare till nästa sida

**HUR OFTA UNDER DE SENASTE 4 VECKORNA HAR DU PÅ GRUND AV DIN ENDOMETRIOS .....**

| | Aldrig | Sällan | Ibland | Ofta | Alltid |
|---|---|---|---|---|---|
| 15. Känt att du inte kan glömma dina symtom? | ☐ | ☐ | ☐ | ☐ | ☐ |
| 16. Känt det som om dina symtom styr ditt liv? | ☐ | ☐ | ☐ | ☐ | ☐ |
| 17. Känt det som om dina symtom tar ifrån dig ditt liv? | ☐ | ☐ | ☐ | ☐ | ☐ |
| 18. Känt dig nedstämd? | ☐ | ☐ | ☐ | ☐ | ☐ |
| 19. Känt dig gråtmild/tårögd? | ☐ | ☐ | ☐ | ☐ | ☐ |
| 20. Känt dig ciändig? | ☐ | ☐ | ☐ | ☐ | ☐ |
| 21. Haft humörsvängningar? | ☐ | ☐ | ☐ | ☐ | ☐ |
| 22. Känt dig på dåligt humör eller lättretad? | ☐ | ☐ | ☐ | ☐ | ☐ |

Var vänlig kontrollera att du har kryssat i ***en ruta för varje fråga***
innan du går vidare till nästa sida

| **HUR OFTA UNDER DE SENASTE 4 VECKORNA HAR DU PÅ GRUND AV DIN ENDOMETRIOS .....** | **Aldrig** | **Sällan** | **Ibland** | **Ofta** | **Alltid** |
|---|---|---|---|---|---|
| **23.** Känt dig våldsam eller aggressiv? | ☐ | ☐ | ☐ | ☐ | ☐ |
| **24.** Känt att du inte kunnat tala om för andra människor hur du mår? | ☐ | ☐ | ☐ | ☐ | ☐ |
| **25.** Känt att andra människor inte förstår vad du går igenom? | ☐ | ☐ | ☐ | ☐ | ☐ |
| **26.** Känt det som att andra människor tycker att du gnäller? | ☐ | ☐ | ☐ | ☐ | ☐ |
| **27.** Känt dig ensam? | ☐ | ☐ | ☐ | ☐ | ☐ |
| **28.** Känt dig frustrerad över att du inte alltid kan använda de kläder du önskar? | ☐ | ☐ | ☐ | ☐ | ☐ |
| **29.** Känt att ditt utseende påverkats? | ☐ | ☐ | ☐ | ☐ | ☐ |
| **30.** Saknat självförtroende? | ☐ | ☐ | ☐ | ☐ | ☐ |

***Nu när du är klar med formuläret, var vänlig och ta med det till ditt mottagningsbesök.***

## 9.4 SF-36 GLOBAL QUESTIONS

**SF-36(tm) Health Survey**

Instructions for completing the questionnaire: Please answer every question. Some questions may look like others, but each one is different. Please take the time to read and answer each question carefully by filling in the bubble that best represents your response.

Patient Name: _____

SSN#: _____ Date: _____

Person helping to complete this form: _____

1. In general, would you say your health is:

   - ❑ Excellent
   - ❑ Very good
   - ❑ Good
   - ❑ Fair
   - ❑ Poor

2. Compared to one year ago, how would you rate your health in general now?

   - ❑ Much better now than a year ago
   - ❑ Somewhat better now than a year ago
   - ❑ About the same as one year ago
   - ❑ Somewhat worse now than one year ago
   - ❑ Much worse now than one year ago

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